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Analysis of Pesticide Residues in Human and Environmental Samples: A Compilation of Methods Selected for Use in Pesticide Monitoring Programs

Association of Official Analytical Chemists
Arlington, VA

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

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This manual provides the pesticide chemist with methodology useful in determining human exposure to pesticides and related industrial chemicals. Methods are also presented for measuring the extent of environmental contamination with these compounds. This manual has been compiled and produced in an effort to promote general acceptance and adoption of uniform chemical methodology of utmost reproducibility and accuracy and to ensure that analytical results can be correlated and directly compared between laboratories. Methods contained in this manual have generally been developed and/or evaluated by this laboratory within the Environmental Toxicology Division. The analytical methodology compiled herein consists of both multiresidue and specific residue procedures. Included also, are miscellaneous topics treating a number of important activities such as the cleaning of laboratory glassware, the preparation of analytical reference standards, and the calibration and maintenance of the gas chromatograph. Several of the methods have been subjected to collaborative studies and have thereby been proved to produce acceptable interlaboratory precision and accuracy. These methods are designated by stars placed at the left of the title in the TABLE OF CONTENTS. Other methods presented are thought to be acceptable but have not been validated by formal interlaboratory collaboration.

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ANALYSIS OF PESTICIDE RESIDUES IN HUMAN AND ENVIRONMENTAL SAMPLES

A COMPILATION OF METHODS SELECTED FOR USE IN PESTICIDE MONITORING PROGRAMS

REVISIONS BY

THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS
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DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This manual provides methodology useful in determining the extent of environmental contamination and human exposure to pesticides and related industrial chemicals. It has been compiled and produced in an effort to promote general acceptance and adoption of uniform chemical methodology of utmost reproducibility and accuracy and to ensure that analytical results can be correlated and directly compared between laboratories. This standardization of data collection will greatly increase our knowledge and understanding of the extent of environmental contamination by pesticides.

F. G. Hueter, Ph. D.
Director
Health Effects Research Laboratory

ABSTRACT

This manual provides the pesticide chemist with methodology useful in determining human exposure to pesticides and related industrial chemicals. Methods are also presented for measuring the extent of environmental contamination with these compounds. This manual has been compiled and produced in an effort to promote general acceptance and adoption of uniform chemical methodology of utmost reproducibility and accuracy and to ensure that analytical results can be correlated and directly compared between laboratories. Methods contained in this manual have generally been developed and/or evaluated by this laboratory within the Environmental Toxicology Division.

The analytical methodology compiled herein consists of both multi-residue and specific residue procedures. Included also, are miscellaneous topics treating a number of important activities such as the cleaning of laboratory glassware, the preparation of analytical reference standards, and the calibration and maintenance of the gas chromatograph. Several of the methods have been subjected to collaborative studies and have thereby been proved to produce acceptable interlaboratory precision and accuracy. These methods are designated by stars placed at the left of the title in the TABLE OF CONTENTS. Other methods presented are thought to be acceptable but have not been validated by formal interlaboratory collaboration.

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INTRODUCTION

The analytical methodology collected in this manual was primarily intended for use by EPA Laboratories conducting analyses of pesticides in various sectors of the environment and by laboratories under contract with EPA to conduct community studies and the monitoring of concentrations of pesticides in the human population.

One of the primary objectives of the Epidemiologic Studies and Monitoring Laboratory program was to establish and maintain, in collaboration with other federal agencies, a broad surveillance and evaluation program concerned with the extent and significance of the contamination of man and his environment by pesticides and their metabolites. To accomplish this goal, data have been continuously obtained on the levels of pesticides and their metabolites in the human population and various elements of the environment. It is important that uniform chemical methodology of utmost reproducibility and accuracy be used by participating laboratories to ensure that analytical results can be correlated and directly compared between laboratories.

A prime responsibility of the Environmental Toxicology Division is to make new and improved analytical procedures available to EPA and related laboratories and to those of state and local agencies working to assess pesticide residues in people and/or environmental media. Thus, the Division serves as a primary facility to provide (1) high purity analytical reference standards, (2) information on analytical quality control, (3) instrumental troubleshooting and calibration, and further (4) to conduct research on analytical methodology for the measurement of residues of pesticides and other toxic residues in human and environmental media.

The analytical methodology compiled herein consists of both multiresidue and specific residue procedures. Included also are miscellaneous topics treating a number of important activities such as the cleaning of laboratory glassware, the preparation of analytical reference standards, and the calibration and maintenance of the gas chromatograph. Several of the methods have been subjected to collaborative studies and have thereby been proved to produce acceptable interlaboratory precision and accuracy. These methods are designated by plus signs placed at the left of the title in the TABLE OF CONTENTS. Other methods presented are thought to be acceptable but have not been validated by formal interlaboratory collaboration.

A numbering system is used in this manual whereby each page bears a date and numbers and/or letters designating the identity of the section and subsection. Additions, deletions and revisions will be distributed to manual holders as they are made available, with each such issuance bearing appropriate section identification and revision date.

The cooperation of scientists using this manual is solicited in helping to improve and update the material. Suggestions and comments based on user's experience will be welcomed. Such suggestions or requests for additional copies of the manual should be directed to:

Director
Environmental Toxicology Division
EPA, Health Effects Research Laboratory
Research Triangle Park, NC 27711

COLLECTION, PRESERVATION AND STORAGE OF SAMPLES

I. GENERAL COMMENTS:

In the procurement, storage, and transportation of samples intended for analysis for pesticide residues, the personnel involved should be aware of some basic considerations to ensure delivery to the analytical chemist of samples that have not undergone degradation of any pesticide present and that have not been contaminated with impurities that might interfere with the analysis.

Although medically trained personnel may be inclined to consider asepsis as the sole requirement, and, while aseptic handling may help ensure freedom from unwanted contamination, there are other far more important considerations. One example is the material of which the sample container is made. Plastics are widely used in the container industries but, although they take preference over glass for many purposes, they should be rigidly avoided as containers for samples that will be examined by gas chromatography. Minute traces of certain of the components of plastics may play havoc in electron capture GLC.

Similarly, ferrous metal containers such as compression lid cans or ointment tins which were used by pharmacies may contain trace impurities that will cause interference in the analysis of GLC.

In general, glass, Teflon, and aluminum foil have been proved to be the most suitable materials to come in direct contact with the sample. Foil or Teflon is generally used as liner material for a bottle or jar cap when the material in the normal cap may contribute impurities. The containers listed in the next subsection are suggested with the foregoing considerations in mind.

II. SAMPLE CONTAINERS:*

A. For tissues:

1. Wide-mouth bottles, glass, 2-1/16 in. high x 1-1/4 in. diam., approx. 1 oz. Owens-Illinois mold number AM-6764. Available from many wholesale glass container distributors. These are generally sold in lots of 1 to 10, 10 to 25, 25 to 50, 50 to 100 and over 100 gross with decreasing per-gross prices for the larger quantities. No caps are included.

These containers are suitable for any autopsy sample not exceeding about 25 grams.

*New containers should be cleaned as described in Section 3, A.

2. The suggested screw caps for the above bottles are metal with paper back foil liners, size 38-400, available in gross quantities from glass container distributors.

B. For blood:

Glass vials in sizes of 45 x 15 mm, 5 ml and 60 x 17 mm, 9 ml. These are available from Arthur H. Thomas Company, Philadelphia, PA under catalog number 9802-G. Caps for these vials are listed as catalog number 2849-A, sizes 13 and 15, respectively. These are molded screw caps with cork back foil liners.

The size should be selected on the basis of the volume of sample drawn and should not be less than 7 ml. Containers with rubber caps should be avoided because of the possibility of contamination from impurities in the rubber. The same warning applies to cork unless a layer of inert material such as foil or Teflon is used on the side contacting the sample.

C. For water:

Water samples may be conveniently taken in glass bottles in which organic solvents are supplied. For example, an emptied hexane or acetone bottle makes an excellent water sample container. The molded screw cap generally has a Teflon liner. If not, a foil liner may be inserted. See Section 10, A for details.

D. For agricultural or environmental media:

Environmental or agricultural samples of 1-lb. or more may be taken in pint, quart or 2-quart size Mason jars. One layer of industrial gauge aluminum foil (0.001 in.) or two layers of regular household grade foil should be used as cap liner. Under no circumstances should the sample material be allowed to come in contact with the paper liner material of the usual metal screw caps.

III. STORAGE OF SAMPLES:

Tissue samples that are to be extracted within 24 hours may be held at normal refrigerator temperature (+2° to +4°C). If extraction is not to be carried out within this time, the samples should be deep frozen at -12° to -18°C.

Blood samples that are to be separated for subsequent analysis of the serum should be centrifuged as soon as possible after drawing. If the serum is to be analyzed within a 3-day period, storage at +2° to +4°C is suitable. If storage is to be for longer periods, it is preferable to deep freeze at -12° to -18°C.

Agricultural or environmental samples that are to be analyzed for organophosphates should be placed in tight containers and stored in deep freeze as soon as possible after sampling unless sample preparation is to be conducted within a few hours. Under no circumstances should extraction be deferred longer than an overnight period, even when the samples are frozen.

IV. SAMPLING, GENERAL:

A subsection on sampling guidelines is included in each method section wherever feasible. In certain sections wherein the sampling and storage may exert a profound influence over the quality of the data obtained from the analysis, the subject is addressed in some detail.

MISCELLANEOUS INFORMATION

CLEANING OF LABORATORY GLASSWARE

In the pesticide laboratory involved in the analysis of samples containing residues in the parts per billion range, the preparation of scrupulously clean glassware is mandatory. Failure to do so can lead to a myriad of problems in the interpretation of the final chromatograms due to the presence of extraneous peaks resulting from contamination. Particular care must be taken with glassware such as Kuderna-Danish flasks, evaporative concentrator tubes, or any other glassware coming in contact with an extract that will be evaporated to a lesser volume. The process of concentrating the pesticide in this operation may similarly concentrate the contamination substance, resulting in extraneous chromatographic peaks that, in extreme cases, may completely overlap and mask the pesticide peak pattern.

Although chemists do not all agree on procedural details in the cleaning of glassware, the majority are in agreement regarding the basic cleaning steps. These are:

1. Removal of surface residuals immediately after use.
2. Hot soak to loosen and flotate most of soil.
3. Hot water rinse to flush away flotated soil.
4. Soak with deep penetrant or oxidizing agent to destroy traces of organic soil.
5. Hot water rinse to flush away materials loosened by deep penetrant soak.
6. Distilled water rinse to remove metallic deposits from the tap water.
7. Acetone rinse to flush off any final traces of organic material.
8. A preliminary flush of the glassware just before using with the same solvent to be used in the analysis.

Each of these eight fundamental steps will be discussed in the order in which they appear above.

1. As soon as possible after use of glassware coming in contact with known pesticides, i.e., beakers, pipets, flasks or bottles used for standards, the glassware should be acetone flushed before placing in the hot detergent soak. If this is not done, the soak bath may

serve to contaminate all other glassware placed therein. Many instances of widespread laboratory contamination with a given pesticide are traceable to the glassware washing sink.

2. The hot soak consists of a bath of a suitable detergent in water of 50°C or higher. The detergent, powder or liquid, should be entirely synthetic and not a fatty acid base. There are very few areas of the country where the water hardness is sufficiently low to avoid the formation of some hard water scum resulting from the reaction between calcium and magnesium salts with a fatty acid soap. This hard water scum or curd would have an affinity particularly for the chlorinated pesticides and, being almost wholly water insoluble, would deposit on all glassware in the bath in a thin film.

   There are many suitable detergents on the wholesale and retail market. Most of the common liquid dishwashing detergents sold at retail are satisfactory but are more expensive than other comparable products sold industrially. Alconox, in powder or tablet form is manufactured by Alconox, Inc., New York and is marketed by a number of laboratory supply firms. Sparkleen, another powdered product, is distributed by Fisher Scientific Company.

   NOTE: Certain detergents, even in trace quantities, may contain organics that will contribute significant background contamination by electron capture detection. For this reason any detergent selected should be carefully checked to ensure freedom from such contamination. The following procedure is recommended:

   Add 25 ml dist. water, previously checked for background contaminants, to a 250 ml sep funnel. Add 1 drop of the liquid detergent (50 mg if in powder form), followed by 100 ml hexane. Stopper funnel and shake vigorously for 2 minutes. Allow layer separation, draw off and discard aqueous layer. Add a pinch of anhydrous Na_2SO_4 to the hexane extract and shake 1 minute. Transfer extract to a Kuderna-Danish assembly fitted with a 10 ml evaporative concentrator tube containing one 3 mm glass bead. Reduce extract volume to ca 3 ml in a hot water bath. Cool, rinse down $ joint and sides of tube with hexane, diluting extract to exactly 5 ml. Stopper tube and shake on Vortex mixer 1 minute. Chromatograph by electron capture GLC and evaluate chromatogram for contaminant peaks.

3. No comments required.

4. The most common and highly effective oxidizing agent for removal of traces of organic soils is the traditional chromic acid solution made up of H_2SO_4 and potassium or sodium dichromate. For maximum efficiency, the soak solution should be hot (40° to 50°C). Safety precautions must be rigidly observed in the handling of this solution. Prescribed safety gear should include safety goggles, rubber gloves and apron. The bench area where this operation is conducted should be covered with lead sheeting as spattering will disintegrate the unprotected bench surface.

   The potential hazards of using chromic sulfuric acid mixture are great and have been well publicized. There are now commercially available substitutes that possess the advantage of safety in handling. These are biodegradable concentrates with a claimed cleaning strength equal to the chromic acid solution. They are alkaline, equivalent to ca 0.1 N NaOH upon dilution and are claimed to remove dried blood, silicone greases, distillation residues, insoluble organic residues, etc. They are further claimed to remove radioactive traces and will not attach glass nor exert a corrosive effect on skin or clothing. One such product is "Chem Solv 2157," manufactured by Mallinckrodt and available through laboratory supply firms. Another comparable product is "Detex" a product of Borer-Chemie, Solothurn, Switzerland.

5, 6, and 7. No comments required.

8. There is always a possibility that between the time of washing and the next use, the glassware may pick up some contamination from either the air or direct contact. To ensure against this, it is good practice to flush the item immediately before use with some of the same solvent that will be used in the analysis.

The drying and storage of the cleaned glassware is of critical importance to prevent the beneficial effects of the scrupulous cleaning from being nullified. Pegboard drying is not recommended as contaminants may be introduced to the interior of the cleaned vessels. Neoprene-coated metal racks are suitable for such items as beakers, flasks, chromatographic tubes, and any glassware then can be inverted and suspended to dry. Small articles like stirring rods, glass stoppers and bottle caps can be wrapped in aluminum foil and oven dried a short time if oven space is available. Under no circumstance should such small items be left in the open without protective covering. The dust cloud raised by the daily sweeping of the laboratory floor can most effectively recontaminate the clean glassware.

Pipet Washing

The efficient washing of pipets offers some special problems. Hand washing performed by soaking pipets in a pan or sink followed by rinsing under running water is highly unsatisfactory, particulary as applied to transfer or volumetric pipets. This procedure does not assure a complete rinse of all surfaces inside the bulb. Therefore, an automatic or semi-automatic washing system is strongly recommended. Self-contained equipment for the entire operation, although available commercially, is quite expensive.

The basic cleaning steps are the same as those listed earlier for miscellaneous glassware, with the exception of the soap wash.

After use, all pipets should be rinsed with an appropriate solvent to remove the bulk of residues remaining in the pipets. The pipets are cleaned by immersion in a chromic acid cleaning solution. For this purpose, the pipets should be in a standard (13.5 x 44 cm) nalgene pipet basket. The entire assembly is submerged in chromic acid in a glass cylinder (16 x 39 cm). After 1-2 hours, the basket of pipets is withdrawn from the chromic acid solution, allowed to drain about 1 minute, and then transferred to a stainless steel washer where rinse water (tap) is run through the washer at the rate of ca.3 minutes per discharge for approximately 1 hour. If piped distilled water is available, seven or eight discharges of this are run through the system to remove all traces of metal contaminants left by the tap water.

A final rinse with acetone, either from a wash bottle or from an overhead syphon bottle, is then applied to each pipet. After draining, a convenient and rapid method of drying is to wrap a bundle of pipets in aluminum foil and place in a drying oven for at least 3 hours, or overnight.

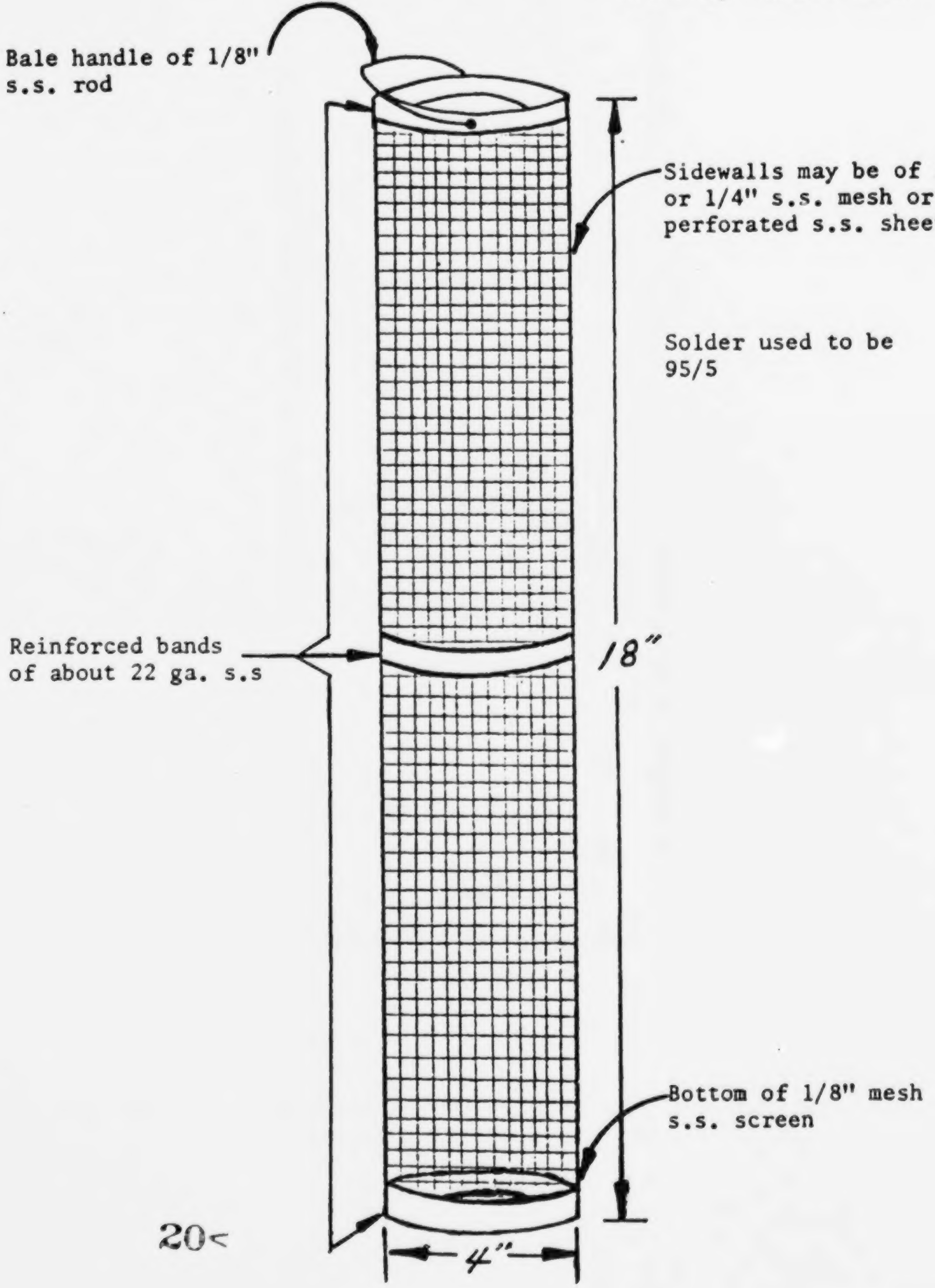
NOTES: (a) Under no circumstances should plastic gloves be worn by personnel during glassware cleaning or handling. It has been determined beyond question that these gloves can most effectively contaminate an entire sinkful of glassware to such an extent that subsequent solvent rinsing may not completely eliminate the contaminants. This is a VERY IMPORTANT precaution.

(b) Drying racks of plastic or plastic-coated metal must be avoided. The latter type of rack may be used, however, after the plastic is scraped from the metal prongs and the rack is cleaned thoroughly with a suitable organic solvent.

FIGURE 1.

PIPET BASKET

Perrine Primate Research Branch
P.O. Box 490
Perrine, Florida 33157


Bale handle of 1/8" s.s. rod
Sidewalls may be of 1/8" or 1/4" s.s. mesh or perforated s.s. sheet.
Reinforced bands of about 22 ga. s.s
18"
Bottom of 1/8" mesh s.s. screen
4"

Solder used to be 95/5

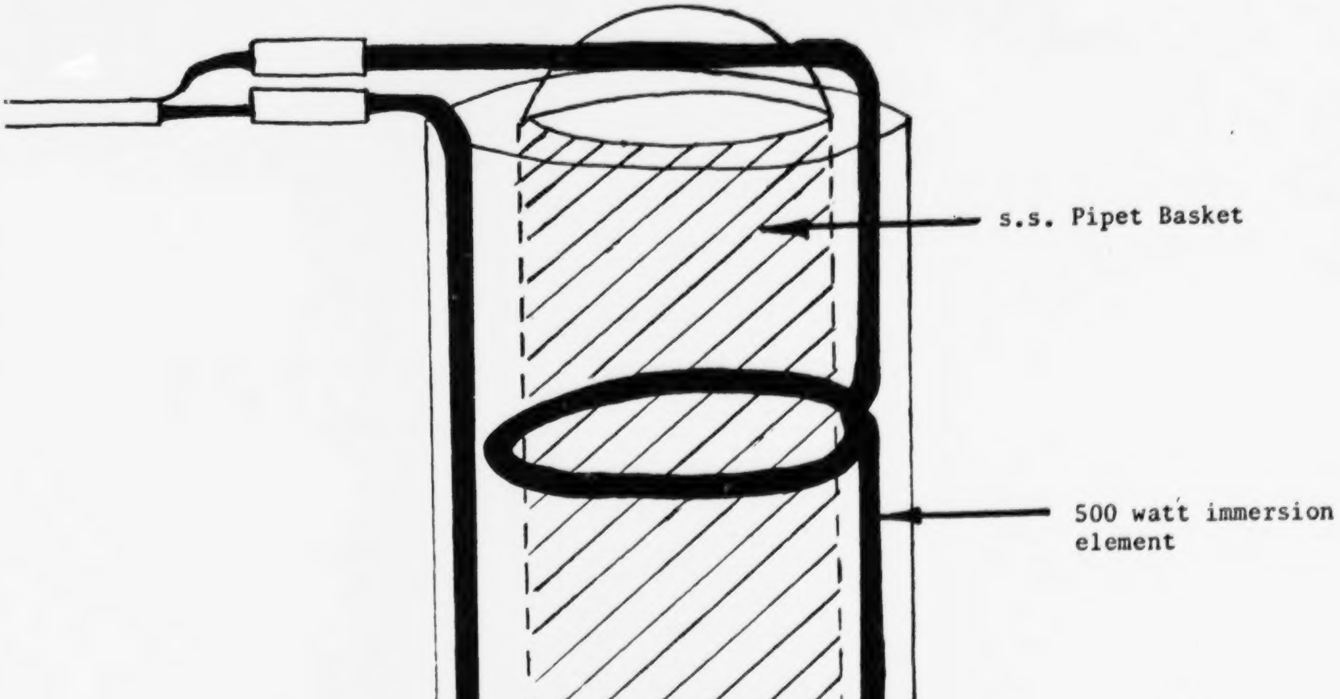

s.s. Pipet Basket
500 watt immersion element
6" x 18" Pyrex glass cylinder

Figure 2. Assembly of pipet washer showing pipet basket inside coiled immersion heater, all contained in Pyrex jar.

MISCELLANEOUS INFORMATION

PREPARATION, STORAGE, AND USE OF PESTICIDE ANALYTICAL STANDARDS FOR GLC

I. EQUIPMENT, SOLVENTS, AND REAGENTS

1. Analytical balance capable of an accuracy of ± 0.05 mg.

2. Spatula, stainless steel.

3. Pipets, Pasteur, disposable.

4. Flasks, volumetric, 25, 50, and 100 ml.

5. Bottles, prescription, ½ oz, 1 oz, or 2 oz, with plastic screw caps. Available from any wholesale pharmacy supply firms. With cap liners, Teflon, sizes 13, 15, 18, and 22 mm, Arthur H. Thomas 2390-H22, H32, H42, and H62.

6. Vial, screw cap, size 1-6, Kontes # K-940100, with Microflex valve # K-749100.

7. Serum bottle, 20-100 ml size, Wheaton # 223742 to # 223747, with Teflon-faced septa # 224168 and seal # 224183.

8. Refrigerator, explosion proof, with freezer across top, capable of maintaining + 4°C in refrigerator section and - 15°C in freezer.

   NOTE: It is definitely preferable to have separate refrigerators for chemicals and sample materials. However, if a laboratory is restricted to one refrigerator, sample materials should be stored in air-tight glass containers to prevent contamination by spillage or airborne vapors from pesticides.

9. Primary pesticide standards. Available in approximately 50 mg quantities to qualified laboratories from the reference standards repository, ETD, HERL, U.S. EPA, Research Triangle Park, NC.

   NOTE: The organophosphorus compounds are subject to a wide variety of oxidation, rearrangement, and hydrolytic reactions. These compounds should be stored in the refrigerator in a large air-tight container (such as a wide-mouth mayonnaise jar) or in a desiccator to minimize moisture absorption and toxic vapor crosscontamination. ALL HANDLING OF THESE STANDARDS SHOULD BE DONE WITH RUBBER GLOVES. SKIN CONTACT BY HIGHLY CONCENTRATED MATERIALS CAN BE FATAL. Samples of organophosphates and metabolites should be equilibrated to room temperature in a desiccator to avoid condensation and possibility of long-term hydrolysis.

10. Sylon-C7 (Supelco) for silanization of empty glass gas chromatographic columns and glass injection inserts.

11. Toluene, isooctane (2,2,4-trimethylpentane), ethyl acetate, or hexane, pesticide quality, distilled in glass.

NOTES: 1. A 10 µl injection of each solvent (except ethyl acetate) should result in a chromatogram with zero background when examined by electron capture GC with system sensitivity adjusted to concur with the criteria outlined in Section 4,A,(4).

2. Isooctane or hexane are both suitable for standard dilution. Isooctane, while more expensive, offers the advantage of a 100°C boiling point and much higher vapor pressure than hexane. The solvent is much less likely to evaporate through long-term leakage around the seal and during repeated bottle openings.

3. Ethyl acetate is not recommended as a final solvent for electron capture GC but may be necessary for preparation of the first or concentrated solution of polar materials.

II. INTEGRITY AND STABILITY OF STANDARDS:

1. Stability of the Solid or Liquid Primary Standard.

Standards that are not in solution are generally stable to chemical decomposition, if kept refrigerated or frozen. Studies done in the past have not shown significant chemical decomposition for time periods in excess of one year. The generally used organochlorines, organophospates, triazines, and carbamates are included in this group. The organophosphate and carbamate standards are subject to hydrolysis reactions. Storage of these compounds in a refrigerated desiccator jar is recommended.

2. Stability of Standard Solutions.

Over the time period of one year, most compounds in hexane, isooctane, or toluene solution are stable to chemical decomposition.

Refrigeration of the standards when not in use is strongly recommended.

Compounds in four classes have been studied. Organochlorine and triazine compounds seem to be stable for long time periods. Most organophosphates and carbamates are also stable. Disulfoton is the only organophosphate that degraded chemically over the period of one year, to the extent of 10% in three months.

The carbamate compounds CDEC and butylate have been found to be unstable as stock solutions and as GC injection solutions. Storage of these solutions for greater than one month is not recommended.

3. Solvent Evaporation Problems.

Most of the problems with standard solution integrity were found to be related to solvent evaporation and the resultant solution concentration. The solvent, the storage temperature, and the storage container are all factors that influence solvent evaporation.

The rate of solvent evaporation from closed containers is related to the vapor pressure of the solvent at the storage temperature. Hexane evaporated 2.4 times faster than isooctane. The vapor pressure of hexane is 3 times the vapor pressure of isooctane. Although the relationship is not perfect, vapor pressure is a better factor to relate to than the boiling point of the solvent. The following is the order of the evaporation rates of the solvents studied:

toluene < isooctane < benzene < methanol << hexane <

acetone < methylene chloride << diethyl ether << petroleum ether

For this reason toluene or isooctane is recommended as the solvent for storage of standards. When solubility or reactivity is a problem, the choice of a solvent should be based partially on the necessary chemical properties and the vapor pressure of the solvents. The use of solvents with high vapor pressures can significantly shorten the shelf-life of standard solutions.

As expected, there is a dramatic difference between solvent evaporation rates at ambient laboratory temperature and in the refrigerator. The life of analytical standard solutions will be lengthened considerably by refrigerated storage when not in use.

The choice of the storage container is a rather critical one. Volumetric flasks are standard storage containers used by many laboratories. However, the standard taper stopper does not form a good seal. The only way to reduce the effect of evaporation is to store a large volume of the standard, thereby reducing the relative solvent loss.

Better containers include prescription bottles with Teflon seals in the cap; these are quite reliable and inexpensive. The graduation mark on the side of the bottle or a piece of label tape can be used to monitor the evaporation of the solvent. Once the solvent evaporation is obvious, the standard is discarded. Two other very good containers are the large volume screw cap vial sold by Kontes and the serum bottle (with Teflon-faced cap) sold by Wheaton. These two containers allow a minimum of solvent evaporation when closed, and they are never opened in use. This reduces the solvent loss dramatically. Once again, a mark or label on the side of the container will serve as a graduation mark. Once the solvent level in the bottle is significantly below the mark, the standard should be discarded.

III. FORMULATION PROCEDURE:

1. Preparation of Concentrated Stock Standard Solutions.

Except for concentrates for special purposes, a concentration of 200 μg/ml is suitable for the common chlorinated and organophosphate pesticides. Ten milligrams of the primary standard, corrected to a 100% purity basis, diluted to 50 ml will provide this concentration (20 mg/100 ml).

Toluene is a suitable solvent for most of the primary standards. β-BHC dissolves readily in toluene, with stirring and a slight application of heat from a hot water bath. For the triazines, the use of ethyl acetate is necessary for the concentrated stock solutions.

The concentrated standards of chlorinated compounds and triazines should maintain uniform strength for a 12-month period at - 10° to - 15°C. The organophosphate standards are less stable than the organochlorines. It is recommended that the concentrated stock solutions of phosphates and carbamates be held no longer than 6 months at - 15°C.

NOTES: 1. Extreme care must be used in the formulation of this standard. If an error is made here, all subsequent dilutions for the life of the standard will be inaccurate. Obviously, all quantitations of samples will be similarly incorrect.

2. Concentrated solutions of CDEC and butylate should not be kept for longer than one month. Very rapid decomposition of the compounds occurs under all conditions.

2. Preparation of Standard Solutions of Intermediate Concentration.

These will be the standards from which the final working mixtures will be prepared. Convenient intermediate concentrations of a number of widely used compounds are given in Table 1.

The solvent for the intermediate standards must be of pesticide quality. Hexane and isooctane are normally used. Isooctane is preferred as discussed in Section 3,B,II.

The intermediate concentration standards of the chlorinated compounds and triazines, if stored in the freezer at - 10° to - 15°C, should be stable for a 12-month period.

The organophosphorus and carbamate intermediate standards should be similarly stored in the freezer. The time limit of these standards should not exceed 6 months.

3. Working Standard Mixtures.

A. Preparation and Storage.

Isooctane is favored as the solvent for the working mixtures since the many repeated bottle openings greatly increase the evaporation and subsequent concentration of standards if a lower boiling point solvent is used.

The attached Table 2 is useful in rapid determination of the aliquot volumes of the higher concentration solutions required to result in given concentrations of the diluted working standards.

The use of standard mixtures of varying concentrations is a necessity for reliable quantitation of unknowns. The degree of peak height variation between sample and standard ideally should not exceed 10%, although variations up to 25% should not result in appreciable error. A simple means of achieving this is to have available working standard mixtures of three concentrations. The suggested mixtures given in Table 3 have proved very useful in the analysis of tissues. Those laboratories conducting analyses on environmental samples may wish to make alterations in the compound content, but the multi-concentration concept should be retained (Miscellaneous Note 5).

The selection of working standard containers and methods of handling and storage are, to some extent, a matter of local preference. Following are two procedures, both of which have proved satisfactory.

1. After the working standard mixtures are diluted in volumetric flasks, they are transferred to prescription bottles with Teflon-lined screw caps, serum vials, or vials with valve tops. These mixtures should be stored in the refrigerator at all times when not in actual use. The organochlorine and organophosphorus working standards should be renewed monthly. With this scheme, large volume glassware should be used.

2. The working standard mixtures are transferred from the volumetric flasks into several small volume (up to 20 ml) containers. When not in use, the standard solutions are kept in the deep freeze. When needed, they are removed from the deep freeze and used. Storage in the refrigerator when not in use is recommended. When the project is completed or the standard has evaporated, a new one should be obtained from the deep freeze and put into use. This option has the advantages of less frequent formulation of working standards and reduced possibility of errors arising from repeated opening of the working standard containers.

Working standards can be used for long periods of time without chemical decomposition. Standards of carbaryl and methiocarb do decompose when exposed to light. These standards should be replaced every 2 months. Disulfoton, CDEC, and butylate decompose rapidly under all storage conditions. Standards of these compounds must be replaced at least every month.

All standards should be replaced when solvent evaporation is obvious when compared to a reference line on the container.

B. Use of Working Standards.

At the start of each working day, after making certain that column operating and instrumental parameters are properly adjusted, it is good practice to make several consecutive injections of standard mixtures to "prime" the column for that day's work. When it has been determined that peak heights for given compounds are constant, the first exploratory injection of an unknown sample extract is made. From this, the chromatographer can now make a number of tentative peak identifications by calculating relative retention values.

The peak height response of some of the compounds in the sample extract may match, within reason, the peak heights resulting from the prior working standard injections. In all probability, certain other compound peaks will not match. The operator will now select from the three working standard concentrations the one that he estimates will produce matching peak heights.

In some cases, it will be found that even the highest concentration mixture will be insufficient to properly quantitate p,p'-DDE and p,p'-DDT. In this case, the sample extract should be quantitatively diluted to a degree that is calculated to produce peaks matching those of the working standards. The pesticide concentrations in the mixtures in Table 3 practically preclude any possibility of violating the detector linearity range of the EC detector when volumes of 5 to 6 μl are injected.

The range of the ^{63}Ni detector is much more restricted than that of the ^{3}H detector in the DC mode. Each detector must be checked for its linearity performance. Improved performance from the ^{63}Ni detector can be obtained in the linearized or pulsed mode.

IV. MISCELLANEOUS NOTES:

1. In addition to the diluted working standard mixtures, each laboratory should maintain a standard of pure p,p'-DDT diluted to 60 pg/μl (the highest concentration of the working mixture). This should be chromatographed daily on each working column to provide current information concerning on-column conversion (generally to p,p'-DDE and/or p,p'-DDD). In case a breakdown peak greater than 3% of the p,p'-DDT is noted, the silanized glass wool plug at the column inlet and the Vykor glass injection insert should be changed. It is most important that the glass injection insert also be silanized. If, after an overnight period of normal operating temperature and carrier gas flow, the situation has not improved, the column should be discarded.

2. If a laboratory has occasion to analyze for endrin, a similar check with an endrin analytical standard should be made weekly. The concentration should be ca.100 pg/μl. The manifestation of endrin breakdown is a depression of peak height response in the main peak accompanied by the formation of two additional peaks. One of these is in the general area slightly later than p,p'-DDT. The other, and largest, peak elutes extremely late, around the methoxychlor

retention area on the OV-17/OV-210 column. If this is observed, the silanized glass wool at the head of the column should be replaced, as well as the glass injection insert.

3. In no case should any attempt be made to dilute standard concentrations to quantitate sample peaks of less than 10% full scale recorder deflection. In view of the sensitivity of which the MT-220 is capable, if all systems are functioning properly, there should be no need to compare peaks with very small areas against each other. The optimum range of peak heights for quantitation lies between 20 and 70% full scale recorder deflection, provided, of course, that the compound concentrations fall within the linear range of the detector.

4. The importance of operating within the limits of the linearity range of the detector cannot be over-emphasized. One means of ensuring this is to operate at a relatively high sensitivity.

5. It is strongly preferable to use the same attenuation setting for standard and sample. If, for any reason, it should appear necessary to use different attenuations, the operator must carefully consider detector linearity limitations and should have prechecked the attenuator linearity. The use of multiconcentration standard mixtures should minimize the need for peak height adjustment by other means.

6. When a new working standard formulation is used for the first time, the peak height response should be carefully compared with the latest chromatograms of the previous mixture. This practice enables the chromatographer to immediately detect any response irregularity, thereby avoiding the use of an incorrect standard for several weeks.

7. It is good practice to standardize injection volumes of standards and sample extracts. A 5 µl injection provides a convenient volume. If alternative volumes are used, they should be restricted to the range of 3 to 8 µl, and each operator should make certain that he can obtain linear response when injecting these volumes.

TABLE 1. SUGGESTED CONCENTRATIONS OF THE INTERMEDIATE STANDARDS OF SOME COMMON PESTICIDAL COMPOUNDS USED IN ELECTRON CAPTURE GLC.

Organochlorine	ng/μl	Organophosphorus	ng/μl
α-BHC	1	Mevinphos	50
β-BHC	2	Phorate	50
Lindane	1	Dimethoate	40
Heptachlor	1	Diazinon	30
Aldrin	1	Methyl Parathion	10
Heptachlor Epoxide	1	Ethyl Parathion	10
o,*p*'-DDE	1	Malathion	20
p,*p*'-DDE	2	Ethion	20
Endosulfan	4	Carbophenothion	10
DDA (Methyl Ester)	[a]	Azinphos Methyl	[a]
Dieldrin	2		
o,*p*'-DDD	2		
Endrin	4		
Perthane	[a]		
p,*p*'-DDD	4		
o,*p*'-DDT	4		
Dilan	10		
Methoxychlor	10		
Tetradifon	20		
Mirex	10		
Chlordane	10		
Toxaphene	[a]		

[a] Final working standard prepared directly from the 200 ng/μl concentrate.

TABLE 2. COMMONLY USED DILUTION VALUES. VALUE IN LEFT COLUMN IS THE AMOUNT (ML) OF CONCENTRATED SOLUTION THAT MUST BE DILUTED TO 100 ML TO ARRIVE AT THE CONCENTRATION VALUE GIVEN IN THE RIGHT COLUMN. VALUE AT HEAD OF EACH COLUMN IS THE CONCENTRATION OF THE STOCK SOLUTION.

1µg/µl		200 ng/µl		20 ng/µl		10 ng/µl	
ml	ng/µl	ml	ng/µl	ml	pg/µl	ml	pg/µl
50	500	50	100	5	1,000	10	1,000
47.5	475	47.5	95	4.875	975	9	900
45	450	45	90	4.75	950	8	800
42.5	425	42.5	85	4.625	925	7	700
40	400	40	80	4.5	900	6	600
37.5	375	37.5	75	4.375	875	5	500
35	350	35	70	4.25	850	4.75	475
32.5	325	32.5	65	4.125	825	4.5	450
30	300	30	60	4	800	4.25	425
27.5	275	27.5	55	3.875	775	4	400
25	250	25	50	3.75	750	3.75	375
22.5	225	22.5	45	3.625	725	3.5	350
20	200	20	40	3.5	700	3.25	325
17.5	175	17.5	35	3.375	675	3	300
15	150	15	30	3.25	650	2.75	275
12.5	125	12.5	25	3.125	625	2.5	250
10	100	10	20	3	600	2.25	225
9.5	95	9.5	19	2.875	575	2	200
9	90	9	18	2.75	550	1.75	175
8.5	85	8.5	17	2.625	525	1.5	150
8	80	8	16	2.5	500	1.25	125
7.5	75	7.5	15	2.375	475	1	100
7	70	7	14	2.25	450	.95	95
6.5	65	6.5	13	2.125	425	.9	90
6	60	6	12	2	400	.85	85
5.5	55	5.5	11	1.875	375	.8	80
5	50	5	10	1.75	350	0.75	75
4.5	45	4.5	9	1.625	325	.7	70
4	40	4	8	1.5	300	.65	65
3.5	35	3.5	7	1.375	275	.6	60
3	30	3	6	1.25	250	.55	55
2.5	25	2.5	5	1.125	225	0.5	50
2	20	2	4	1	200	.45	45
1.5	15	1.5	3	0.875	175	.4	40
1	10	1	2	0.75	150	.35	35
0.5	5	0.5	1	0.625	125	.3	30
				0.5	100	0.25	25

continued

TABLE 2. (CONTINUED)

4 ng/μl		2 ng/μl		1 ng/μl	
ml	pg/μl	ml	pg/μl	ml	pg/μl
20	800	35	700	50	500
18.75	750	32.5	650	45	450
17.5	700	30	600	40	400
16.25	650	27.5	550	37.5	375
15	600	25	500	35	350
13.75	550	22.5	450	32.5	325
12.5	500	20	400	30	300
11.25	450	17.75	375	27.5	275
10	400	17.5	350	25	250
9.375	375	16.25	325	22.5	225
8.75	350	15	300	20	200
8.125	325	13.75	275	17.5	175
7.5	300	12.5	250	15	150
6.875	275	11.25	225	12.5	125
6.25	250	10	200	10	100
5.625	225	8.75	175	9.5	95
5	200	7.5	150	9	90
4.375	175	6.25	125	8.5	85
3.75	150	5	100	8	80
3.125	125	4.75	95	7.5	75
2.5	100	4.5	90	7	70
2.375	95	4.25	85	6.5	65
2.25	90	4	80	6	60
2.125	85	3.75	75	5.5	55
2	80	3.50	70	5	50
1.875	75	3.25	65	4.5	45
1.75	70	3	60	4	40
1.625	65	2.75	55	3.5	35
1.5	60	2.5	50	3	30
1.375	55	2.25	45	2.5	25
1.25	50	2	40	2	20
1.125	45	1.75	35	1.5	15
1.	40	1.5	30	1	10
.875	35	1.25	25	0.9	9
.75	30	1	20	.8	8
.625	25	.75	15	.7	7
0.5	20	0.5	10	.6	6
0.25	10			.5	5
0.125	5				

TABLE 3. SUGGESTED MIXTURES FOR QUANTITATION OF COMMON CHLORINATED COMPOUNDS IN TISSUES.

SERIES		Standard concentration in pg/μl 1	2	3
A. (6%)	HCG	2.5	5	10
	γ-BHC	5	10	20
	β-BHC	7.5	15	30
	Aldrin	5	10	20
	Oxychlordane	7.5	15	30
	Heptachlor epoxide	7.5	15	30
	_t_-Nonachlor	7.5	15	30
	_p,p_'-DDE	40	80	160
	_o,p_'-DDT	15	30	30
	_p,p_'-DDD	20	40	80
	_p,p_'-DDT	25	50	100
	Mirex (if suspected)	40	80	160
B. (15&)	Aldrin	5	10	20
	Dieldrin	10	20	40
	Endrin (if suspected)	12.5	50	100
*C	α-BHC	5	10	20
	δ-BHC	5	10	20
	Heptachlor	5	10	20
	Aldrin	5	10	20
	_o,p_'-DDE	10	20	40
D.	Aroclor 1254	125	250	500
	Aroclor 1260	125	250	500

*This series contains only those compounds that are rarely found in tissues.

MISCELLANEOUS INFORMATION

GENERAL PURITY TESTS FOR SOLVENTS AND REAGENTS

In general, the solvents used in pesticide residues by GLC must be of very high purity. If the laboratory intends to use the purchased solvents without redistilling, materials bearing the manufacturer's designation of "pesticide quality, distilled in glass" should be purchased. Even with this designation, each lot must be checked for assurance of freedom from any impurity that may have escaped the manufacturer's quality control. If drum lots of technical or commercial grade solvents are bought, distillation through an all-glass still is practically mandatory.

I. TEST FOR SUBSTANCES CAUSING INTERFERENCE IN ELECTRON CAPTURE GLC:

Electron capture GLC requires solvent that is free of substances causing detector response at the electrometer attenuation normally used in analytical work. Place 300 ml of the solvent in a specially cleaned 500 ml Kuderna-Danish concentrator fitted with a 3-ball Snyder column and a 10 ml evaporative concentrator tube. Evaporate in a hot water bath to 5 ml. Inject 5 μl of this concentrate into the gas chromatograph and allow enough time for elution of any peak equaling the retention time of the latest eluting compound of possible interest to the laboratory. This would generally be the retention area of Guthion. If no peaks elute at the retention sites of the compounds of interest, and adjacently eluting peaks are not sufficiently large to create a partial overlapping with pesticides, the indication is favorable for the purity of the solvent. If any peak(s) of greater than 2% FSD elute at the retention sites of one or more of the pesticides of interest, the solvent would create problems in identification and quantitation and would not be acceptable. The electrometer attenuation should be that currently in use for sample analysis.

It may be possible to remove the contaminants by distillation through an all-glass still. However, there is no certainty of this because some organic materials may codistill with the solvent and still be present in the distillate.

II. TEST FOR SUBSTANCES CAUSING PESTICIDE DEGRADATION:

Solvent impurities not detected by the above procedure may cause degradation and loss of pesticides during analysis. Solvents should be tested for suitability by carrying known amounts of both chlorinated and organophosphate pesticides through the method in the absence of any sample substrate. Solvents containing oxidants may cause noticeable loss of organophosphate pesticides, especially carbonphenothion.

III. REAGENTS:

A. Acetonitrile - Some lots of reagent grade acetonitrile are impure and require redistillation. Vapors from impure CH_3CN turn litmus paper blue when moistened paper is held over mouth of storage container.

B. Ethyl Ether - Must be free of peroxides. The test is outlined in Section 5, A, (1) under REAGENTS.

NOTE: The ether from one manufacturer is sold in a metal can. A polyethylene cap is provided for resealing the can during use. It has been determined that contaminants from the polyethylene cap can prove most troublesome, particularly when the 200 ml of 15% ether fraction is concentrated down to 1.0 ml in Method 5, A, (1).

C. Sodium Sulfate, Sodium Chloride and Glass Wool - These materials used in the cleanup procedure, even of reagent quality, frequently cause interfering peaks. This is so prevalent that it is good practice to Soxhlet extract with the solvent(s) to be used in the method and dry in 130°C oven before use. Fifty extraction cycles are usually sufficient to remove the impurities.

MISCELLANEOUS INFORMATION

EVALUATION OF QUALITY OF FLORISIL

I. INTRODUCTION:

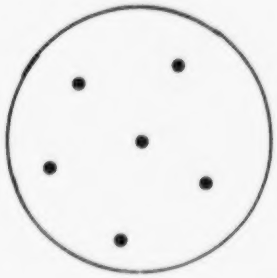
Florisil, PR grade, is available from a number of distributors or from the Floridin Division of the Pennsylvania Glass Sand Company. It is packed in various size units up to 20 Kilos.

The "PR" grade used for pesticide residue analysis is checked at the producer's laboratory for activity characteristics to ensure uniformity. However, these characteristics may vary slightly from batch to batch, and, therefore, each new lot purchased should be evaluated by the user to determine the elution and recovery characteristics for the pesticides of interest in the user's laboratory.

If the material is purchased in fiber drums lined with polyethylene, the evaluation sample may be drawn from the drum(s) in accordance with the following guidelines: Immediately following the evaluation, the material should be transferred from the drum(s) to glass containers with foil-lined lids to avoid the possibility of contamination of the Florisil by trace quantities or organic contaminants in the polyethylene drum liner.

II. SAMPLING:

A drum is sampled by taking six plugs from top to bottom with a 36 in. x 1 in. grain trier. The approximate plug pattern should be as shown in the following sketch:



The trier plugs from all drums are placed in a single container and are mixed thoroughly. Three elution columns are prepared as described in Section 5,A,(1), and the prepared columns are stored in a 130°C oven at least overnight.

NOTE: If the normal procedure of the laboratory is to pack the columns immediately before use, the prepacking of columns for overnight activation may be avoided. In this case, the flask of Florisil should be held in the 130°C oven at least 16 hours before use.

III. STANDARD MIXTURES:

Prepare the following standard mixtures, using hexane or isooctane as the solvent.

		Standards pg/µl		
Compound	Florisil	6%	15%	50%
Hexachlorobenzene	20	20		
Lindane	20	20		
Heptachlor	20	20		
Aldrin	40	40		
Heptachlor epoxide	60	60		
Dieldrin	40		40	
Endrin	100		100	
o,p'-DDT	80	80		
p,p'-DDD	50	50		
p,p'-DDT	80	80		
Mirex	100	100		
Diazinon	2500		2500	
Methyl parathion	250		250	
Malathion	400			400
Ethyl parathion	400		400	
Carbophenothion	250	250		

The "Florisil" standard is used to elute through the Florisil columns. The 6%, 15%, and 50% standards are used as quantitation standards during gas chromatographic analysis.

IV. FLORISIL ELUTION:

All glassware used in this procedure should be meticulously cleaned; chromic acid is recommended for thorough cleaning.

1. Remove the prepacked columns from the oven so they may cool before use.

2. Read and record the percentage relative humidity in the room.

3. Place a beaker or flask under each column and prewet the packing with 50 ml of petroleum ether.

   NOTE: From this point on throughout the following elution process, the solvent level should not be allowed to go below the top of the Na_2SO_4 layer.

4. With 5 ml volumetric pipets, transfer 5 ml of the standard mixture onto duplicate columns and 5 ml of hexane onto the third column as a control.

5. Place 250 ml Kuderna-Danish assemblies with 10 ml graduated tubes under each column and commence elution with 100 ml of diethyl ether-petroleum ether (6:94 v/v) at a rate of 5 ml/minute. Measure the 100 ml portion of elution solvent in a graduated cylinder and apply to the column when the liquid level just reaches the top of the Na_2SO_4 layer. At the instant the liquid level of the first 100 ml of eluting solvent just reaches the top of the Na_2SO_4 layer, place a second 250 ml K-D assembly under the column. Quickly add another 100 ml of eluting solvent and let this solvent pass through the column.

6. Continue elution with 200 ml of diethyl ether-petroleum ether (15:85 v/v) in two separate 100 ml portions. Collect the eluate in two separate K-D assemblies identified as 200-300 ml and 300-400 ml.

7. Continue the elution with diethyl ether-petroleum ether (50:50 v/v), following the same procedure of collecting the 100 ml increments that are designated as 400-500 ml and 500-600 ml.

8. Place the 18 K-D assemblies containing the 100 ml eluate increments on a 100°C steam bath and evaporate the contents to ca. 2-5 ml.

9. Evaporate remaining solvent under a nitrogen stream to 1 ml, remove from the bath, and let cool. Dilute the samples containing pesticides with hexane to exactly 5 ml. Do not dilute the control samples.

10. Stopper the evaporative concentrator tubes and mix on a Vortex mixer for 1 minute.

    NOTE: Another way to evaluate the blank is to elute the column with the full 200 ml volumes of the 6 and 15% solvents. Then concentrate the eluate to 1.0 ml before injection.

V. GAS CHROMATOGRAPHY:

1. With a column of 1.5% OV-17/1.95% OV-210 installed in the instrument, prime the column as described in Section 4,A and equilibrate the instrument.

    NOTE: Use only the column designated, on which the compounds in the respective mixtures will overlap only minimally.

2. Make 5 µl injections of the fractions collected from each of the three columns and the 6%, 15%, and 50% standards.

    NOTE: In case of off-scale peaks or peaks of less than 10% FSD, make appropriate attenuation adjustment for both standards and eluates. For valid comparisons, measure both the standards and the samples at the same attenuation.

VI. CALCULATIONS AND TABULATION:

1. Measure all peak heights from original standards and eluate increments.

2. Based on peak heights measurement for each compound, calculate the percentage of the compound appearing in each 100 ml increment and in the original standard.

Example:	Lindane,	0-100 eluate, peak ht.	30 mm
		100-200 eluate, peak ht.	60 mm
		Original standard	98 mm

    Percentages in the 0-100 eluate = $\frac{30}{30 + 60} \times 100 = 33\%$

3. Compute the elution recovery by dividing the sum of the combined eluate peak heights by the peak height of the original standard.

    Working from the same data given above:

    Recovery = $\frac{30 + 60}{98} \times 100 = 92\%$

With the possible exception of aldrin, the recoveries of the chlorinated compounds should fall in the range of 90 to 105%. Aldrin may not exceed 80%. Some of the compounds may not yield high recoveries. For example, trithion may yield no higher than 40% recovery under certain conditions, as outlined in Subsection VIII, Note 1.

4. Tabulation of results may be made on a form comparable to Table 1. The decision of acceptance or rejection of each lot is based on a consideration of the elution pattern and recovery efficiency of the pesticides of interest in the program.

5. Evaluation of the control columns should also be taken into account before the Florisil is accepted. There should be no peaks in the chromatograms that would influence pesticide quantitation.

VII. STORAGE OF FLORISIL:

It is imperative that the Florisil be transferred from the shipping drums into glass jars as soon as possible after the lot is evaluated and judged acceptable. The drums are lines with polyethylene, which may contribute unwanted contamination over a period of time.

Glass jars that have been found suitable for storage are available from certain glass container distributors. A suitable jar bears Owens-Illinois mold No. C-3122, with 100-400 finish, packed in cartons of six jars. Metal screw caps with coated paper liners are used.

The jars may be washed by mechanical dishwashers, and then rinsed with distilled water and acetone. After the jars are thoroughly dried, the Florisil may be transferred with a 2 lb aluminum sugar scoop previously washed and acetone rinsed. The net content of each jar, when filled within 1/2 inch of the rim with Florisil, is ca. 2 lb. A square of aluminum foil is crimped over the rim of the jar, and the cap is screwed on tightly. Each jar is labeled with the lot number and is now ready for storage.

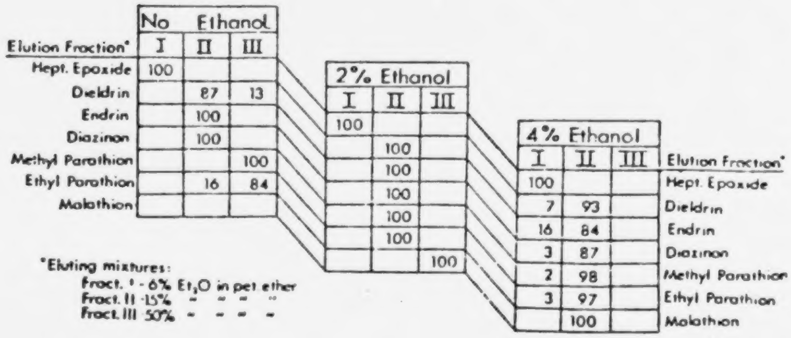
VIII. NOTES:

1. Factors influencing the recovery efficiency, particularly of certain organophosphorus compounds, include the presence of impurities in the petroleum ether and the presence of peroxides in the diethyl ether. This is discussed in more detail in the MISCELLANEOUS NOTES of Section 5,A,(1).

2. The polarity of the elution solvents exerts a profound effect on the selective elution of a number of compounds. The diethyl ether must contain 2% v/v of ethanol to obtain compound elution patterns comparable to those shown in Table 1. The following chart

demonstrates the effects resulting from altering the amounts of ethanol in the diethyl ether.

The effects of polarity variation of eluting solvent in Florisil partitioning of 7 pesticides. Absolute diethyl ether mixed with 0, 2, and 4% absolute ethanol.



	No Ethanol			2% Ethanol			4% Ethanol		
Elution Fraction*	I	II	III	I	II	III	I	II	III
Hept. Epoxide	100			100			100		
Dieldrin		87	13		100		7	93	
Endrin		100			100		16	84	
Diazinon		100			100		3	87	
Methyl Parathion			100		100		2	98	
Ethyl Parathion		16	84		100		3	97	
Malathion						100		100	

*Eluting mixtures:
Fract. I - 6% Et_2O in pet ether
Fract. II -15% " " " "
Fract. III 50% " " " "

3. If possible, the Florisil oven should be reserved only for adsorbents and not used for general laboratory purposes. Any spillage or introduction of organic materials inside the oven may contaminate the Florisil (or other adsorbent materials) and result in a profusion of contaminant peaks when the final eluates are chromatographed.

4. In the assessment of extremely low concentrations of pesticides in samples, it is not uncommon to concentrate the fraction eluate(s) to as little as 1.0 ml rather than 5.0 ml. This may pose a problem in background contamination not evident in the 5.0 ml concentrate. Scrupulous care must be taken in the cleaning, storage, and handling of the glassware. When significant contaminant peaks are obtained with EC detection, the operator is often inclined to fault the Florisil, which is a possibility; however, it is far more common to find that the actual problem is contaminated glassware.

TABLE 1. ELUTION PATTERNS AND RECOVERY DATA FOR FLORISIL, LOT # 2854, BY METHOD SECTION 5,A,(1) (MANUAL OF ANALYTICAL METHODS)
FLORISIL COLUMN PREPACKED AND HELD IN 130°C OVEN AT LEAST 24 HOURS BEFORE USE
RELATIVE HUMIDITY IN LABORATORY 65 %

	ELUTION INCREMENTS (ml)						
Compound	6% Fraction		15% Fraction		50% Fraction		
	0 - 100	100 - 200	200 - 300	300 - 400	400 - 500	500 - 600	Recovery, %
α-BHC	100						97
β-BHC	100						95
Lindane	100						96
Heptachlor	100						91
Aldrin	100						100
Hept, Epox.	78	22					105
Dieldrin			85	15			96
Endrin			89	11			99.6
p,*p*'-DDE	100						97
o,*p*'-DDT	100						99.6
p,*p*'-DDT	100						90
Ronnel	100						93
Methyl Parathion			47	53			103
Malathion					100		99
Ethyl Parathion			78	22			96
Diazinon			100				83
Trithion	100						43

Numerical values represent the percentage of each compound eluting in the given fraction.

MISCELLANEOUS INFORMATION

LIMITS OF DETECTABILITY

The Analytical Chemistry Committee, comprised of representatives from the Community Studies laboratories, the Perrine Chemistry Section and the Division of Community Studies, Chamblee, Georgia, met in December 1969. Among the topics discussed was that of the lower limits of detectability of pesticidal compounds in human tissues.

The Committee recognized the necessity for the establishment of such limits so that data from all laboratories would be reported in a comparable manner.

The two tissues considered were blood and adipose tissue. The limit recommendations were based upon data from quality control check samples, recommendations from individual project chemists, and the experience of the Committee members. The recommendations do not imply toxicological significance, reflecting only the apparent analytical potential within the confines of the currently prescribed methodology. It is entirely possible that further studies may indicate the advisability of revising the limits. For the present, the established limits are as follows:

Compound	Conc. in ppb Adipose	Serum
α-BHC	10	1
Lindane	10	1
β-BHC	20	1
Aldrin	10	1
Heptachlor	10	1
Heptachlor Epoxide	10	1
o,p'-DDE	20	1
p,p'-DDE	10	1
Dieldrin	10	1
Endrin	20	2
o,p'-DDT	20	2
p,p'-DDD	20	2
p,p'-DDT	20	2

GAS CHROMATOGRAPHY-ELECTRON CAPTURE INSTRUMENT

In this section, operating instructions of a specific nature are intended to apply to the model Tracor 220 or 222 gas chromatograph manufactured by Tracor, Inc., Austin, TX. This instrument may be equipped with a DC detector containing ^{63}Ni or 130 mc ^{3}H. However, many of the following guidelines are broadly applicable to a wide range of chromatographic instruments.

I. FLOW SYSTEM:

The flow system consists of the entire system through which nitrogen gas will flow, from the common point of entry at the exit of the filter drier branching to (1) the purge line running through the purge rotameter and flow controller thence through the detector, and (2) the carrier flow line running through the rotameters, the flow controllers, and the column, thence through the transfer line into the detector.

It is essential that no leaks exist anywhere in the flow system. Even a minute leak will result in erratic baselines with the ^{3}H detector. The ^{63}Ni detector will be even more seriously affected. Leaks can be detected by the application of "Snoop" at all connections or by spraying the connection with Freon MS-180 with the instrument operating and observing recorder response. Spray short squirts close to the connection. Do not spray around the detector or injection port.

II. DETECTOR:

This subject is covered in detail later in Section 4, A, (3).

III. ELECTROMETER:

To ensure proper daily operation of the unit, set the attenuators to the OFF position and zero the recorder. Set the output attenuator at x1 and observe the baseline. A steady baseline with less than 1% noise is considered good. A check should occasionally be made of the electrometer electronic zero. Instructions for doing this may be obtained from the Electronics Shop at Research Triangle Park, NC.

Zero and bucking controls should operate "smoothly" and should not cause erratic recorder response.

Check the "maximum" polarizing voltage available. If at least -130 v DC is not available on the rear panel, it is quite possible that the power printed circuit board (PCB) is not functioning properly and damage or noisy operation will result from continued use.

IV. TEMPERATURE PROGRAMMER:

The operator should be certain this unit is functioning properly. When the unit is operating properly, the column over temperature should not show appreciable deviation. If the temperature fluctuation is excessive, baselines will cycle and, in all probability, retention measurements will be erratic.

In an emergency situation, a 10 amp variable transformer (Variac or Powerstat) may be used as a temporary measure. Constant use of this device is not advised as it does not operate on temperature demand, but simply supplies a fixed voltage to the heating elements. Therefore, oven temperature will vary with any changes in line voltage and room temperature.

V. PYROMETER:

The batteries of this unit should be checked monthly to be sure they are delivering full voltage under load. This can be done easily with a voltmeter set on the 3-volt range and shunting a 1 megohm resister across the voltmeter leads to constitute a load. If the voltage under this test situation falls below the rated voltage for the battery, replace battery. The battery contacts should also be cleaned by spraying with Freon MS-180 and wiping with dry cloth. To prevent shorts, it is recommended that electrical glass cloth tape be wound around each end of the battery at positions where the clamps hold the battery in place.

A hint of inaccurate pyrometer operation may be obtained by switching to one of the unused sensors and observing the readout. If the reading is more than 5°C from room temperature, faulty operation is suggested. This is suggested as a daily check to prevent straying gradually into grossly inaccurate temperature readings. Before final readings are made, gently finger tap the pyrometer frame in the area around the set screw.

VI. MISCELLANEOUS:

A. Septums - There are a number of different types available, ranging from the inexpensive plan black (or gray) silicone rubber to the sophisticated "sandwich" type selling at a significantly higher price.

Excellent results have been reported using the blue silicone rubber material marketed by Applied Science Laboratories as their "W" series.

The 13 mm precut septums are available in lots of 100 under catalog number W-13. The same material listed as "Type W" is available in sheets of 12 in. x 12 in. About 400 13-ml septums can be cut from this sheet with a No. 9 cork borer making the price per hundred syptums about half that of the precut septums.

B. Column "O" Rings:

The conventional column "O" rings are of heat-resistant silicone rubber, and must be used with brass ferrules. The "O" rings are available in varying sizes from all suppliers of gas chromatography accessories. The chromatographer may prefer to use Teflon ferrules instead of brass. If these are used, no "O" rings are required.

C. Prepurified nitrogen gas shall be used for the DC mode of operation. Argon containing 5% methane (P-5 Mix) is recommended for linearized ^{63}Ni detectors. This is piped to the instrument through a filter drier of molecular sieve, 1/16" pellets, Linde type 13X. Before the filter drier is charged with fresh molecular sieve, the interior of the drier should be rinsed with acetone, and the drier unit should be placed in a 130°C oven for at least 1 hour. The bronze frit should be rinsed with acetone and flamed. After filling, the unit should be heated at 350°C for 4 hours with a nitrogen flow of ca.90 ml/minute passing through the unit. If the activated unit is to be stored for a period of time before use, the ends should be tightly capped.

NOTE: Argon-methane cannot be used for operation of the flame photometric detector. Separate carrier gas systems must be used on instruments equipped with linearized ^{63}Ni electron capture and flame photometric detectors.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE
COLUMNS

I. SPECIFICATIONS:

Column material shall be of borosilicate glass, 6 feet (1.8 m) long, 1/4 in. (6 mm), o.d., 5/32 in. (4 mm) i.d. Because off-column injection will be used, one side of the column shall be 1 in. longer than the other. The Swagelok nut, ferrule and silicone "O" ring are assembled as in Fig. 4. Complete column specifications for the Tracor MT-220 gas chromatograph are given in Fig. 11.

II. COLUMN SELECTION:

There is a wide variety of column packing materials in the marketplace, some of which are entirely suitable for use in pesticide analysis, and others which are of limited value. In general, the columns selected as a "working pair" should be significantly different in polarity and in their compound elution characteristics. One pair that has proved very useful is given as A and C below. B provides another alternative. The peak elution patterns for 13 chlorinated pesticidal compounds on each of these columns are shown in Figures 1 through 3.

A. 1.5% OV-17/1.95% OV-210 - liquid phases premixed and coated on silanized support, 80/100 mesh.

B. 4% SE-30/6% OV-210 - liquid phases premixed and coated on silanized support, 80/100 mesh.

C. 5% OV-210 - coated on silanized support, 100/120 mesh.

III. PACKING THE COLUMN:

Make certain the column is actually 6 feet long. A paper template tacked to the wall is a convenient and quick means of checking this. For off-column injection in the Tracor Model 220 or 222, one column leg should be 1 in. shorter than the other.

With a china marking pencil, place a mark on the long column leg 2 in. from the end. Place a similar mark 1-1/8 in. from the end of the short leg.

Add the packing to the column through a small funnel, ca.6 in. at a time, and bounce the column repeatedly on a semihard surface.

Rapid tapping up and down the column with a wooden pencil will promote settling of the packing. The packing is added until it reaches the mark on each leg and it is found that additional tapping will not produce any further settling.

> NOTE: This operation should be done with great care, tapping the column a sufficient length of time to be certain that no further settling is possible by manual vibration. The use of mechanical vibrators is not advised because the packing can be packed too densely, thus, introducing the possibility of an excessive pressure drop when carrier gas flow is started.

Pack silanized glass wool into both ends of the column just tightly enough to prevent dislodging when carrier gas flow is started.

> NOTE: If the glass wool is manipulated by hand, the hands should be carefully prewashed with soap or detergent, rinsed and dried. This minimizes the possibility of skin oil contamination of the glass wool.

IV. COLUMN CONDITIONING:

The column is conditioned, or made ready for use, in two operations: (1) by heat curing, and (2) by silylation treatment.

1. Heat Curing.

A Swagelok fitting is attached to the inlet port at the top of the oven. This is comprised of a 1/4-in. Swagelok to AN adapter, part number 400-A-4ANF, connected to a 1/4-in. male union, part number 400-6.

Before assembling, the bore of the union must be drilled out with a 1/4-in. drill and burnished with a rat-tailed file so that it will accept the 1/4-in. o.d. column glass.

The short column leg is attached to the above fitting, with the end of the long leg venting inside the oven. The nut, ferrule, and "O" ring are assembled as shown in Fig. 4. Make sure the nut is tight, because the "O" ring will shrink during the curing period, thus allowing carrier gas to escape.

> NOTE: The outlet ports leading to the transfer line should be sealed off during the conditioning period to prevent traces of column effluent from seeping through to the detector. This is easily done by assembling a 1/4-in. Swagelok nut on a short piece of 6-mm glass rod with ferrule and "O" ring.

2. Silylating Treatment.

Treatment with a silylating compound such as Silyl 8 serves to block active adsorption sites, particularly in a new column, thereby somewhat improving efficiency and resolution characteristics. The most drastic effect is in the improvement of endrin response and the near elimination of on-column breakdown of endrin. Silyl 8 is available in 1- and 25-ml septum capped bottles from the Pierce Chemical Company, P.O. Box 117, Rockford, Illinois 61105.

At the end of the prescribed heat curing period, adjust the oven tempset and carrier gas flow controllers to the appropriate settings to give the approximate recommended operating parameters for the given column. While the temperature is dropping, open the oven door and, wearing heavy gloves, retighten the Swagelok nut which will invariably loosen during heat curing. Close door and allow oven temperature to equilibrate. Make four consecutive injections of 25 µl each of Silyl 8, spacing the injections ca 1/2 hour apart. Allow at least three hours for the final injection to elute off the column before proceeding.

NOTES:

1. Syringe used for Silyl 8 injections should be used for no other purpose, and should be flushed with benzene immediately after use to avoid plugging of the needle.

2. It is strongly advised that Silyl 8 be discarded after one year and that fresh material be ordered; observations in the Editors' laboratory have indicated some troublesome side effects in electron capture GLC arising from the use of old Silyl 8.

V. EVALUATION OF COLUMN:

Shut down oven and carrier gas flow, remove column from special fitting, remove fitting from inlet port, and connect column to detector, making sure that nuts are securely tightened. Replace Vykor glass injection insert with a clean one and install a fresh septum. Make certain that the stainless steel retainer for the insert is reinstalled with the slotted end up. Upside down installation will permit the escape of carrier gas. After septum nut is screwed down by hand, a little further tightening with pliers helps ensure gastight septum installation. Raise oven temperature and carrier gas flow to the exact values given in Table 1 for the appropriate column. The oven temperature must be monitored by some means other than the built-in pyrometer, either with a precalibrated dial face thermometer with the stem inserted through the oven door, or with a mercury thermometer pushed down through an unused injection port. DO NOT RELY WHOLLY ON THE INSTRUMENT PYROMETER.

Check the carrier gas flow rate using the sidearm buret device sketched in Fig. 4 (a) attached to the purge exit of the detector. DO NOT RELY ON THE INSTRUMENT ROTAMETER in adjusting the carrier flow. Allow an overnight period for complete equilibration of the column-detector system at normal operating parameters of temperature and carrier flow.

> NOTE: If two columns are connected to the same detector, the carrier flow to the column not in use should be shut off while the flow rate through the column in use is being measured. Likewise, the purge line flow controller should be closed. The unused column flow should also be kept at zero while determining the background current.

After overnight equilibration, recheck the oven temperature and carrier gas flow rate. You are now ready to assess the performance characteristics of the column, and this should definitely be done before attempting to use the column for routine work.

Run a background current profile at the normal operating parameters for the given column, with the purge line flow controller set at 4. Detailed instructions are given under Subsection 4,A,(3) DETECTOR. The BGC profile is particularly important in providing an assessment of detector behavior as affected by the column. It is presumed that a BGC profile was run on the same detector within a few days from the time of the present profile, so that the expected level of background current may be compared to the level obtained in the present test. If the present level falls far short of that expected, either the detector itself is faulty or the column is exerting an adverse effect on the detector. The column influence may be roughly determined by allowing several hours more for equilibration and repeating the BGC profile. If an increase in BG current is obtained, additional checks are made until no further increase is noted. A typical BGC profile is shown in Fig. 5.

If the detector foil is new and the BG current is at a high level, it is acceptable practice to set the polarizing voltage at 85% of the full BGC profile. However, this practice is not reliable with an older, partially fouled detector. A more reliable method is to run a polarizing voltage/response curve as described in Subsection 4,A,(3) OPTIMUM RESPONSE VOLTAGE. A polarizing voltage/response curve is shown in Fig. 6.

The operator should now be ready to chromatograph some standard mixtures to evaluate the efficiency, resolution, compound stability and response characteristics of the new column. A mixture that has proved very useful in assessing performance is made up as follows, the concentration of each compound stated in terms of picograms per microliter:

α- BHC	10	Hept. Epoxide	30	*o,p'*-DDD	80
β- BHC	40	*p,p'*-DDE	40	*p,p'*-DDD	80
Lindane	10	Dieldrin	50	*o,p'*-DDT	90
Heptachlor	10	Endrin	80	*p,p'*-DDT	100
Aldrin	20				

The mixture is made up in isooctane, and, if kept tightly stoppered in the deep freeze, it should be usable for a year or more, strictly for column evaluation purposes but not for quantitation. Its value for column evaluation lies in the number of very closely eluting peaks. The chromatograms in Figs. 1, 2, and 3 were obtained from this mixture.

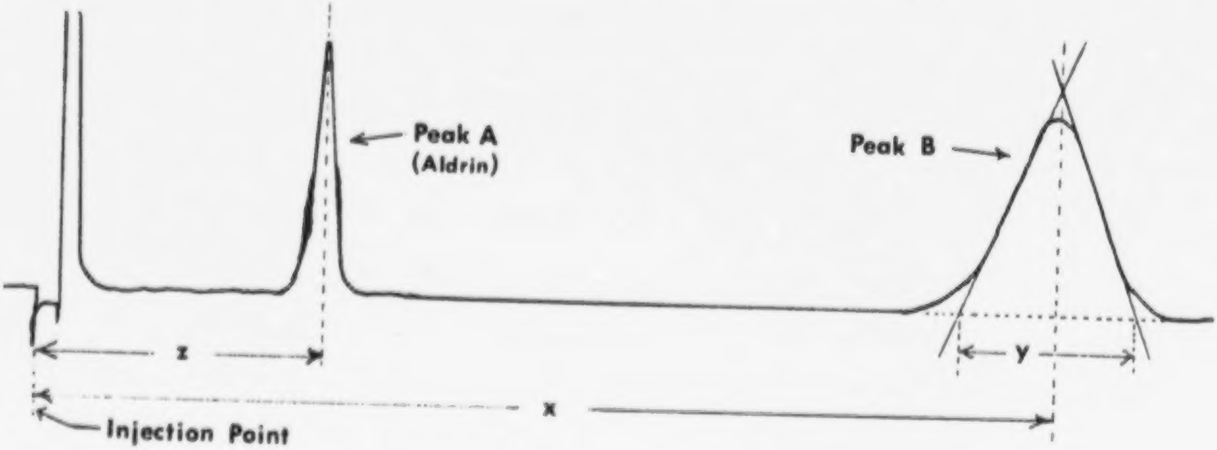
Several things about the new column can be learned from chromatographing this mixture.

1. The column efficiency can be determined from computation based on the *p,p'*-DDT peak. The equation is given on next page.

   If the computed efficiency is less than 2,700 theoretical plates, *and* if the resolution between peaks is not comparable to that shown by Figs. 1, 2 or 3, the indication is clear that something has gone wrong in the preparation, conditioning and/or use of the column, provided of course that high quality column packing was used in preparation of the column.

2. Compute the relative retention value for *p,p'*-DDT and compare this value to the values given in Table 2, a, b, or c. This should enable the operator to determine his precise column temperature and to relate this to the readout from pyrometer and outboard thermometer.

3. Compute the absolute retention in minutes for *p,p'*-DDT from the equation given below and compare with the value given on the chromatogram furnished with the packing. If the value varies by more than 2 minutes from the value stated in Table 2, it is indicated that (1) one or both operating parameters are off,



$$N = \left(\frac{4x}{y}\right)^2$$

$R_{x_1} = \frac{x}{6.35}$ (At 1/4-in./min chart speed)

$R_{x_2} = \frac{x}{8.38}$ (At 1/3-in./min chart speed)

$R_{x_3} = \frac{x}{12.7}$ (At 1/2-in./min chart speed)

$R_{x_4} = \frac{x}{16.76}$ (At 2/3-in./min chart speed)

$R_{x_5} = \frac{x}{25.4}$ (At 1-in./min chart speed)

$RRT_A = \frac{x}{z}$

Where N = column efficiency in total theoretical plates.

Rx_1, x_2, etc. = absolute retention, in minutes, for peak B.

RRT_A = retention ratio, relative to aldrin, for peak B.

x,y,z = measurements in millimeters.

or (2) column is not 6 feet long, or (3) the density of the packing is not comparable.

a. If the absolute retention is less than the table value by more than 2 minutes, the oven may be running too hot or the carrier flow may be running too high, or both; the column may be packed too loosely, offering less surface area of coated support; the column may not be a full 6 feet in length.

b. Conversely, if the absolute retention value is high, the indications may be: a low column temperature, a low carrier flow, a column packed too densely, or some combination of two or more of these factors.

Based on the chromatograms of the evaluation mixture, a decision generally can be made as to the potential quality of the column. If, after making slight adjustments in the carrier gas flow rate, characteristics of efficiency absolute retention and peak resolution do not compare reasonably well with the chromatograms and data furnished by Table I and Figures 1, 2 or 3, it is inadvisable to proceed further with the column. For example, if an efficiency value of over 2,700 theor. plates cannot be obtained on a new column, it is unlikely that the column would ever improve to much over 3,000 T.P. On the other hand, if the new column yielded 3,000 T.P., it is probable that it would improve to 3,300 or 3,500 T.P. after becoming "seasoned".

Assuming that a favorable indication is obtained from the mixture chromatograms, the next evaluation step is to determine the compound breakdown characteristics of the column. This may be done by injections to produce peak heights of 50 to 60% FSD. The DDT breakdown should not exceed 3%. The endrin response and breakdown characteristics may be determined similarly. This breakdown should not exceed 10%.

NOTE: This breakdown percentage is calculated by adding up the peak areas of main peak and breakdown peak(s). This value divided into the peak area value for the breakdown peak(s) x 100 is the breakdown percentage.

VI. MAINTENANCE AND USE OF COLUMN:

A column that is used and maintained properly should provide service for many months. It is difficult to make precise time estimates because of the variables present in different laboratories. Data from a column performance survey showed one laboratory using the two working columns 3-1/2 months for an estimated number of 1,300 injections of predominantly fat extract. Another laboratory indicated their two columns to be at least a year old, and each had been subjected to an estimated 3,000 injections of blood extract. Neither laboratory's columns gave any indication of deterioration. In fact, the laboratory injecting fat predominantly was included in the group showing superior overall column performance.

The Vykor glass injection insert used in off-column injections serves as a trap to prevent a high percentage of dirty material from befouling the front end of the column. If this insert is not changed frequently, however, column performance characteristics can be significantly altered. When a sufficient amount of residue collects in this insert, lowered efficiency, compound breakdown, peak tailing, and depressed peak height response become evident. The changing of this insert should be on a daily basis if sample extracts of any kind are being injected.

The effects of Silyl 8 conditioning do not persist indefinitely. Any laboratory with an interest in endrin detection may find that resilylation may be necessary at intervals to be determined by weekly monitoring for breakdown.

A certain amount of extraneous matter is eluted through the glass insert and lodges in the glass wool plug at the column inlet. Indications from the survey mentioned above were that those laboratories changing the glass insert daily could go for long periods of time without changing the column plug. Daily compound conversion monitoring provides a constant check on the need for changing the glass wool plug.

When the column is idle overnight or over weekends, a low carrier flow of ca 25 ml per minute through the column is advised. Simultaneously, a purge flow of ca 25-30 ml through the detector is also advised. If a column is out of the instrument longer than 2 or 3 days, reconditioning is advised wherein the column is not connected to the detector, but is allowed to vent into the oven under a carrier flow of ca 60 ml per minute at a temperature ca 25° above the prescribed operating temperature.

An erratic and noisy baseline can indicate leaks in the column connections or at some other point in the flow system, starting at the injection septum and on to the detector inlet. If the baseline has been stable and first became erratic upon installation of a new column, the probability of loose column connections is indicated.

If any laboratory has trouble obtaining performance characteristics equal to those indicated by the chromatograms and data furnished in Table I and Figure 1, 2 or 3, every effort should be made to pinpoint the trouble and correct it. If the foregoing instructions are followed with no deviations, trouble should not be experienced.

VII. SOURCES OF COLUMN PACKINGS

The question is often raised concerning the advisability of a laboratory making its own column packing or buying it precoated from a commercial producer. If a laboratory staff member has developed the expertise to make consistently high quality column packing, this is the less expensive route. However, it should be noted that few individuals possess this "knack". Coupled with the science, there is a degree of art in the formulation of small batch lots of quality column packing. Lacking this expertise, the laboratory would be well advised to purchase precoated packing, prescribing a set of quality specifications with the purchase order. The specifications should include:

(1) A statement listing a group of pesticidal compounds such as the list given on page 5 of this section along with the required retention values, relative to aldrin, at a given column temperature. This is of particular importance for mixed liquid phase packing to ensure the proper proportion of liquid phase components.

(2) A statement of minimum efficiency in terms of the total theoretical plates in a 6-foot column as computed by the method shown on page 6 of this section.

(3) A stated range of absolute retention, in minutes, for a given compound such as p,p'-DDT when column is operated at given parameters of temperature and carrier gas velocity.

(4) A statement prescribing maximum decomposition limits for such compounds as endrin and p,p'-DDT under prescribed operating parameters.

VIII. MISCELLANEOUS NOTES:

1. The carrier flow through the unused column should not be carried any higher than is required for positive pressure. Detector response is seriously affected by running both columns simultaneously at normal operating velocity. For instance, in a series of observations with a pair of nearly identical low-load columns in the oven, the peak height response for aldrin is reduced ca 25% when the off-column is carried at 70 ml/min, the same flow at which the on-column is being operated.

2. An obvious, but sometimes overlooked, point arises when only one column is installed in the oven. The transfer line commonly used is the dual type that conveys column effluent from the two-column outlet ports to the single detector. When one column is removed, its outlet port must be plugged or else a massive leak will be created. One easy means of doing this is to slip swagelok fittings and an "O" ring on the end of a short piece of 1/4 in. o.d. glass rod and install in the unused outlet port.

3. Columns shorter than 6-ft. are generally suitable for chromatography of specific, late eluting compounds as retention time can be shortened for greater work output. However, for multiresidue analysis on samples of unknown composition, the shorter columns are not advised. Shorter columns are less efficient and therefore yield much poorer peak resolution. This can be an important factor in peak identification.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR

Straight DC polarizing voltage should be supplied to the detector from either an outboard power supply unit or a strip on the back of the electrometer. Provided the column and all electronic circuits in the various modules of the instrument are functioning properly, the degree of sensitivity in the electron capture mode relates most probably to the condition of the interior of the detector. As radioactivity in the foil decreases, so does sensitivity of the system. Measurement of the background current gives an indication of the condition of the detector and should be run on a new or overhauled detector. Subsequent periodic measurements should be made to provide up-to-date information on the performance of the detector as influenced by the condition of the foil or by any other effects such as column bleed or contaminated carrier gas.

I. BACKGROUND SIGNAL PROFILE:

1. Zero recorder and electrometer in the normal manner.

2. With a well-seasoned column such as OV-17/QF-1 in the instrument, set input attenuator on 10 and output attenuator on 256.

   NOTE: The given attenuation values apply to electrometer Model E2. If the dual channel, solid state unit is used, an equivalent setting would be 10^2 x 128.

3. Set column and detector temp. and carrier flow rate to the levels prescribed for the column in use. Apply ca 70 ml/minute of purge gas.

4. Set OUTPUT POLARITY switch to the polarity opposite of that used in normal operation.

5. Reduce polarizing voltage to zero using control on power supply unit or in front of electrometer, and adjust bucking control of electrometer to permit zeroing of pen on chart paper.

6. Set chart speed on 1/4 inch per minute, start chart drive, and allow about 1/2 inch horizontal trace.

7. Advance polarizing voltage control to 5 volts and allow sufficient time for trace to level off; then repeat for 10, 15, 20, 25 volts and so on, until a voltage value is reached which produces no further recorder deflection.

Generally, a new detector or one with a new tritium foil should be expected to produce a response of 60 to 80% full scale deflection. With aging, as the response level approaches about 30% FSD, a replacement of the foil is indicated. Figure 5 shows a background signal profile on a detector in constant use for 2 months. At the time of original installation, the background signal profile produced 68% FSD.

II. OPTIMUM RESPONSE VOLTAGE:

It is important that a correct polarizing voltage be supplied to the detector to achieve maximum peak response with minimal overshoot. An incorrect voltage can result in (1) full potential sensitivity of the detector not being utilized, or (2) a strong overshoot in the peak downstroke which makes for difficult quantitation of peaks. The optimum response voltage is determined as follows:

1. Upon completion of the background signal profile, reset OUTPUT POLARITY switch back to normal operating position and set polarizing voltage control to the voltage that produced ca 60% of the total BGC profile.

   NOTE: If you are fairly certain that the optimum polarizing voltage will fall in some fairly high range, i.e., 20 to 25 volts, time can be saved by starting about 7 volts under the expected optimum polarizing voltage.

2. Set oven and detector temperatures and carrier flow rate to the prescribed operating levels for the column in use, and allow system to equilibrate.

3. Set attenuators on the values appropriate for the condition of the detector.

4. Adjust bucking control to zero recorder pen.

5. Inject an aldrin standard in quantity known from current operation to produce a peak about 1/2 full scale at the attenuation being used.

   NOTE: The volume injected must be carefully measured and should not be less than 5 μl.

6. Repeat injection to obtain peaks from increments of 2.5 volts, i.e., 15, 17.5, 20, etc., until two peaks show less height than that obtained on the highest peak.

NOTE: Occasionally a new detector will require only around 7 volts, and it may be found that the 2.5-volt intervals result in too much change in response. In this case, it may be advisable to use 1-volt intervals to set up the response curve.

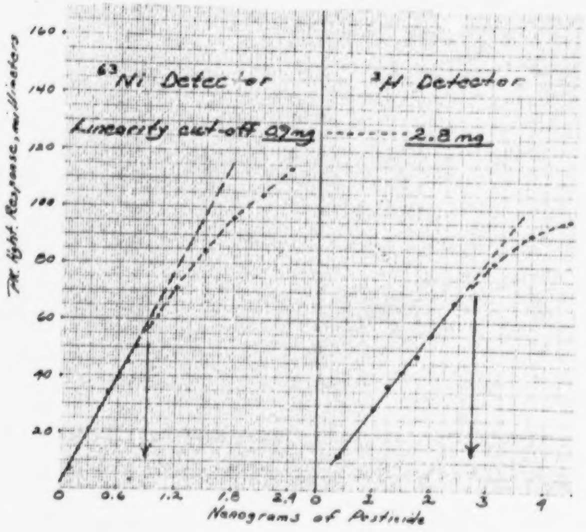
7. Taking the exact peak height values, measured in millimeters, plot a peak height vs voltage curve on linear graph paper (Figure 6). Usually the optimum polarizing voltage is the next voltage interval higher than the voltage producing the greatest response, in other words, a point on the downslope of the curve. However, if appreciable peak overshoot is evident at this voltage, it may prove desirable to set polarizing voltage slightly higher to minimize overshoot at some expense in response. The arrow in Figure 6 indicates the voltage selected in this particular case.

III. DETECTOR LINEARITY:

In making chromatographic runs for quantitation, it is mandatory that compound concentration be within the linearity range of the detector. As this characteristic may change with the age and use of the detector, standard curves for pesticides of interest should be run periodically to provide up-to-date linearity information. In most cases, operation at an output attenuation setting of 10 x 8 (or 16) on the E2 electrometer or 10^2 x 8 (or 16) on the SS will preclude the possibility of violating the linear range of the detector. If samples are diluted so that quantifiable peaks are produced at these settings, the large errors resulting from calculations based on nonlinear response can be avoided.

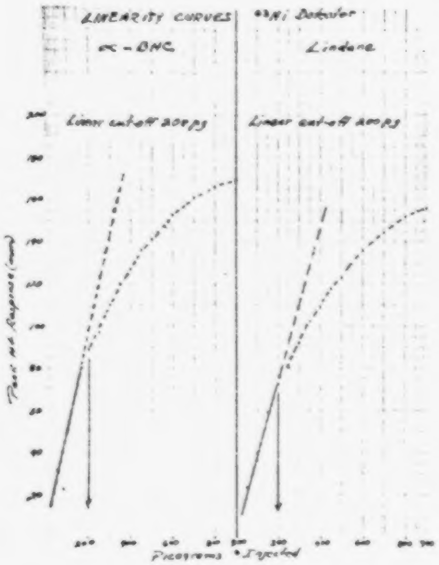
The ^{63}Ni detector operated in the DC mode is far more restrictive in linearity characteristics than the ^{3}H detector. The linearity curves in Figure 6 illustrate the comparative linearity of ^{63}Ni and ^{3}H detectors. Linearity curves should be run frequently and, most important, on each new detector or on one subjected to overhaul.

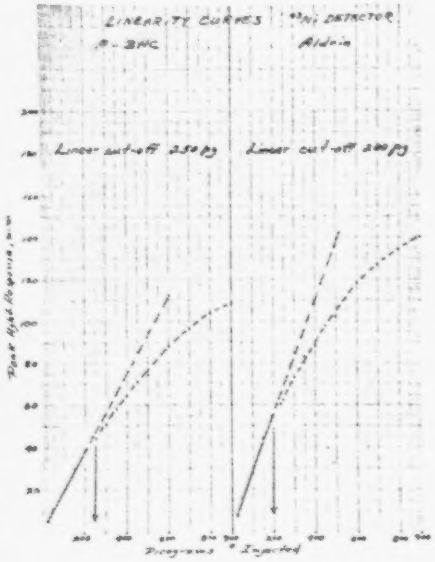
Figure 6

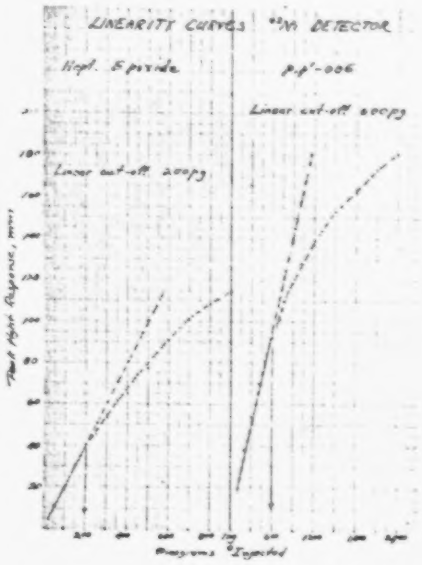


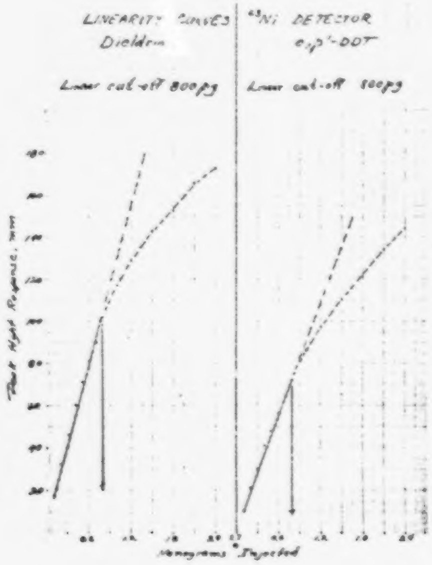
Linearity curves for p,p'-DDD using ^{63}Ni and ^{3}H detectors

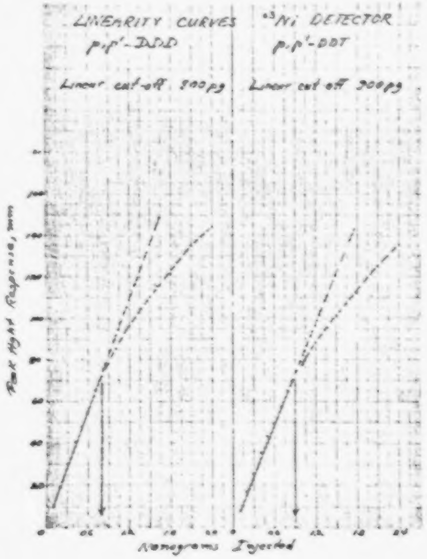
Figure 6 (con't)











IV. TRACOR LINEARIZED ^{63}Ni ELECTRON CAPTURE DETECTOR:

The linearized EC detector is operated in the constant current pulsed mode. As electron capturing compounds enter the detector, the polarizing pulse frequency changes in order to keep the detector cell current and standing current constant. The signal generated is amplified and displayed on a strip chart.

Details of set-up, operation, theory of operation, GC parameters, detector profiles, and circuit alignment can be found in the Operation Manual 115314B supplied with the detector.

Advantages of the linearized EC detector include:

1. Wider linear range than the DC mode - a linear response of ± 5% is obtainable with argon-methane (95:5 v/v) carrier gas from 5×10^{-12} to 5×10^{-8} gram of lindane. In some cases, operation to 2×10^{-7} gram of lindane within the above linearity has been achieved.
2. Can be operated in a somewhat "dirty" (contaminated) condition with less loss of performance.
3. Can tolerate more contaminants in the carrier gas.
4. Gives a generally narrower solvent front.
5. Has sensitivity comparable to the DC mode.

Disadvantages of the linearized EC detector include:

1. Argon-methane carrier gas is more expensive.
2. Nitrogen carrier gas can be substituted for argon-methane, but the linear response range is reduced for higher concentrations with the nitrogen carrier.
3. Linearity must be checked over each concentration range used for actual samples.
4. Electronic alignments can be difficult.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

CHROMATOGRAPHY OF SAMPLE

When the chromatographic system has been idle for a number of hours, such as overnight or over the weekend, it is generally necessary to "prime" the column before quantitation may be attempted. The first early morning standard injection will frequently show relatively poor response. The second and third injections will usually improve the response to a constant level. This "priming" may be done by successive injections of a dilute working standard mixture or it may be accomplished by one injection of a highly concentrated mixture. One laboratory has reported excellent results with the latter system and, if other laboratories obtain comparable results, considerable daily "priming" time may be saved. The suggested priming mixture is given below; the concentration values are in nanograms per microliter.

Lindane--------0.5
β-BHC----------1.5
Aldrin---------0.5
Hept. Epox.----1.0
p,p'-DDE-------1.0
Dieldrin--------1.0
o,p'-DDT--------1.5
p,p'-DDD--------1.5
p,p'-DDT--------1.5

Forty microliters of this mixture is injected. If this one-shot system is used, a special syringe should be set aside and used solely for this purpose. Under no circumstances should the same syringe be used for routine injections.

In the early morning the priming may be conducted while the other daily instruments checks are being made. If more than one column in the instrument is to be used, the priming may be done simultaneously. After the priming mixture has eluted off the columns, the carrier flow should be carefully adjusted for the working column using the bubble device shown in Figure 4(a). The chromatograph should now be ready for the first working standard injection.

A sample extract concentration of 10 ml from a 5 gram sample contains the tissue equivalent of 0.5 milligram per microliter. A 5 µl injection of this extract (2.5 milligrams of sample) into an EC detector of average sensitivity should easily produce quantifiable peaks at pesticide concentrations of at least 0.1 ppm, provided that instrumental attenuation is appropriately adjusted.

1. With the working column in the instrument, adjust column and detector parameters as prescribed in Table 1. If another column is in the oven, set a positive carrier gas pressure of not more

than 20 ml/min on this alternative column or, if preferred, leave carrier flow at zero on a column of high thermal stability. Set attenuation at an estimated appropriate sensitivity.

NOTE: The specified GLC instrument has a high sensitivity potential provided that all modules are functioning properly. It is important to take full advantage of this potential by avoiding low sensitivity attenuation. With a new detector foil, low sensitivity attenuation may be necessary, but as the BGC decreases, this practice, while resulting in a stable looking baseline, requires injections of relatively high sample concentration to produce quantifiable peaks. This tends to promote faster fouling of column and detector than would result from injections containing less sample material. This is particularly important when injecting eluate from the 15% ethyl ether/pet ether extract from fat. If all instrumental modules are functioning properly it should be possible to obtain a noise level not exceeding 1% of full scale at an attenuator setting of 10 x 8. If 10 x 8 should not meet this specification, then 10 x 16 should definitely produce an acceptable noise level. In the event that the electrometer noise level at 10 x 16 should exceed 1% full scale, some electronic trouble shooting may be indicated.

2. If a column is used for which an RRT/Temp. table is available (Tables 2, a, b, or c), the procedure for tentative peak identification in an unknown is relatively simple and requires far less time than traditional "cut and try" methods. First it is necessary to establish the prevalent true column temperature. This is determined by chromatographing a standard mixture containing aldrin and p,p'-DDT. Other compounds eluting earlier than p,p'-DDT may be included, but their presence may be irrelevant for this mission. Calculate from the chromatogram the RRT_A of p,p'-DDT, then by scanning horizontally across the column opposite p,p'-DDT on the table, locate the RRT_A value which most closely matches the calculated value. The actual column temperature can now be obtained by reference to the top or bottom of the table.

3. Inject 5 µl of the sample extract as a preliminary run to determine whether all peaks are on scale and are of quantifiable peak height. If off-scale peaks are observed, make an estimated dilution of a portion of the extract and reinject.

NOTE: Injections of volumes less than 5.0 µl should be avoided in quantitation. The possibilities for error are greatly enhanced by low volume injections.

4. Calculate the RRT_A values for all peaks appearing on the sample chromatogram(s). By vertically scanning the appropriate temperature column on the table, the calculated RRT_A values may be compared with table values to obtain tentative peak identifications.

5. The information derived from Step 4 above should provide the operator with sufficient intelligence re tentative compound identities and estimated concentration ranges to facilitate the selection of an appropriate working standard mixture for precise quantitation. Subsequent injections of standards will then be carried out bearing in mind that (1) peak heights between sample and standard should vary not more than 25%, (2) the concentration of all compounds must fall well within the linear range of the detector, and (3) no peak of less than 10% FSD should be quantitated.

6. At this point the task of compound identification is incomplete, and confirmation must be conducted on an alternative column of completely different polarity (see Section 4,A,(2). The chromatographer must be constantly aware that artifact peaks may be obtained with one column which may have identical RRT_A values with certain pesticidal compounds; also that a number of pesticidal compounds may have identical or near identical RRT_A values on a given column. The last point must be carefully considered in the selection of an alternative column that will resolve such overlaps.

MISCELLANEOUS NOTES:

1. It is desirable to use standard mixtures with the component pesticides at three concentration levels. This will enable the operator to select a mix whose concentrations will fall within the linear range of the detector and have a peak size comparable to the unknown peaks.

2. The height of sample and standard peaks should preferably vary by not more than 25%. It is sometimes alleged that this point is of no consequence provided both standard and sample are within the linear range of the detector. In theory this is true, but like many theoretical postulations, the fact does not necessarily follow the theory. For example, the theory does not take into consideration minor response variations arising from injection error and/or instrumental sources. It can be easily demonstrated that a response variation of as little as 3 mm in peak height can result in a final error of 20 to 25% when a 13 mm sample peak is calculated against a 130 mm standard peak.

3. Electrometer attenuation should be adjusted to obtain a minimum sensitivity level of a peak of 50% FSD resulting from the injection of 100 picograms of aldrin.

4. Quantitation by referencing sample peaks against a standard curve may be an acceptable practice provided that certain limitations are carefully considered. It must be recognized that repetitive injections of certain sample extracts may gradually depress response characteristics of the GLC system. When this occurs, a curve established from a standard or mixture of standards at 9 AM on a given day may be worthless by 11 AM on the same day. This possibility must be monitored by interspersing standard injections continually throughout the work day. In view of this requirement, the construction of a curve becomes a superfluous and unnecessary task as quantitative referencing can be made against the interspersed standards.

5. At this point, detailed evaluations are made of all chromatograms. If there is reason to suspect any peak identification or quantitation, instrumental controls should be switched over to alternative column for further scrutiny. The isomers of BHC, o,p'-DDE, and o,p'-DDT frequently pose identification problems. If such identification problems are present and cannot be confidently resolved by any of the three prescribed columns, further confirmatory work is required by electrolytic conductivity detection and/or by TLC.

GAS CHROMATOGRAPHY-ELECTRON CAPTURE

QUANTITATION AND INTERPRETATION

There are several methods for quantitating chromatographic peaks. While we are not partial to any particular method, it is desirable in a system of laboratories providing data to a central point that some degree of uniformity be specified.

The preferred method of calculation is somewhat dependent on peak shape. The major categories of peak shapes are: (1) Tall, narrow, and symmetrical, generally illustrated by a p,p'-DDT peak from a clean extract, (2) Overlapping peaks where the overlap is estimated not to obscure the peak height, (3) Unsymmetrical peaks such as are commonly encountered in an uncleaned extract.

Broadly speaking, quantitation methods recommended for the various types of peaks are:

I. PEAK HEIGHT:

A. Early eluting peaks, tall and narrow.

B. All peaks on the trace where there are no obscuring overlaps and where peaks are tall, symmetrical, and fairly narrow (Figure 7).

II. PEAK HEIGHT X WIDTH AT HALF HEIGHT:

A. Separated, symmetrical, and fairly wide peaks (Figure 8).

III. TRIANGULATION OR INTEGRATION:

A. Separated unsymmetrical peaks, or peaks on sloping baseline (Figure 9). Triangulation should not be attempted on very narrow peaks. Extreme care must be taken in the construction of the inflectional tangents and in measurements.

IV. INTERPRETATION:

Although this subject is listed last in this section devoted to EC GLC, it is far from being the least important. An excellent performance in all other areas may be nullified if the chromatograms are not properly interpreted.

The electron capture detector, being non-specific, responds to any electron-capturing materials in addition to pesticides in the final extract being chromatographed. For this reason, the task of interpretation is one requiring careful study of the data and the application of sound judgment. The presence of chromatographic peaks which precisely match the absolute and relative retention values of those of certain pesticides does not necessarily indicate the indisputable presence of those pesticides. For example, it is not uncommon to observe peaks from human tissue extract with retention characteristics precisely the same as α-BHC and/or o,p'-DDE. Confirmation by ancillary techniques has never supported the electron capture detector indications, however. In one instance methyl parathion was reported in a blood sample. Had the individual conducting the interpretation exercised sound judgment, it should have been immediately apparent that the presence of the parent compound of parathion in body fluids other than gastro-intestinal would be a near impossibility.

The chromatographer must recognize that quite often peaks are obtained from a given sample substrate on one GLC column by electron capture detection, the retentions of which strongly suggest certain pesticidal compounds. If, based on experience, these particular compounds are not likely to be present in the sample material, some further confirmation is required. This may be done by (1) using an alternative column and electron capture detection, (2) applying electrolytic conductivity detection, (3) thin-layer chromatography, (4) chemical derivatization, (5) gas chromatography-mass spectrometry, or (6) high performance column liquid chromatography.

TABLES AND FIGURES

Tables and figures in this section will assist the analyst in column selection and operation by providing retention data on compounds for tentative identification of unknown peaks in a multiresidue analysis (see Subsection 4,A,(4).

Figures 1 through 3d are typical chromatograms of a 13-compound mixture, each column operated at a temperature and carrier gas flowrate providing maximum efficiency with reasonable retention times. Since the parameters may differ widely, comparisons of retention parameters on different chromatograms should not be made. For example, retention times in Figures 3a and 3d are not directly comparable because chart speeds were 0.5 and 0.25 inch per minute, respectively.

Because the elution pattern of compounds with DEGS differs from the patterns from most other columns used in pesticide analysis, the DEGS column (Figure 3a) often proves useful in resolving problems relating to peak identification. Field reports, however, warn of bleed problems with DEGS and suggest that the DEGS column not be used for a "working" column since it is likely to foul the detector. For the brief periods the column is used for confirmations, bleed effects are not apt to be troublesome.

Stationary phases that are chemically similar and give similar elution patterns are listed in the following table. For example, the elution pattern for a given pesticide mixture for 5% DC-200 will be very similar to that from 5% SE-30 or OV-101.

Stationary Phases Commonly Used in Pesticide Analysis

No. Designation		Chemical Name	Similar Phases
1.	DC-200	Methyl silicone	OV-1, OV-101, SE-30, SP-2100, DC-11, SF-96
2.	QF-1	Trifluoropropyl methyl silicone	OV-210, SP-2401
3.	SE-30	(See No. 1)	
4.	SE-52	Methyl silicone, 10% phenyl substituted	OV-3
5.	SF-96	(See No. 1)	
6.	XE-60	Cyanoethyl methyl silicone	OV-225
7.	OV-17	Methyl silicone, 50% phenyl substituted	SP-2250
8.	OV-7	Methyl silicone, 20% phenyl substituted	-------
9.	OV-210	(See No. 2)	
10.	DEGS	Diethyleneglycol succinate	none

TABLE 1. CONDITIONING, OPERATION PARAMETERS AND PERFORMANCE EXPECTATIONS FOR 6-FT. X 1/4-IN. O.D. COLUMNS OF PRECOATED PACKINGS, 3% DEGS INCLUDED SOLELY AS A CONFIRMATORY COLUMN, NOT FOR ROUTINE USE.

Parameters	1.5% OV-17 / 1.95% OV-210	4% SE-30 / 6% OV-210	5% OV-210	3% DEGS
Liquid phase(s)	Silicone OV-17 Silicone DC QF-1 (FS1265)	Silicone SE-30 Silicone DC QF-1 (FS1265)	OV-210 Trifluoromethylpropyl Silicone	DEGS Stabilized Diethylene Glycol Succinate (Analabs C4)
Solid Support	Chromosorb W, H.P. or Gas-Chrom Q 100/120 mesh	Chromosorb W, H.P. or Gas-Chrom Q 80/100 mesh	Chromosorb W, H.P. or Gas-Chrom Q 100/120 mesh	Gas-Chrom P 80/100 mesh
Heat Curing Temp °C Time Hours	245 48 (minimum)	245 72 (minimum)	245 48 (minimum)	235 20 (exact)
Operating Temp °C	200	200	180	195
Detector Temp °C (tritium)	205	205	205	205
Carrier Flow ml/minute	50-70	70-90	45-60	70-90
Elution Time for p,p'-DDT Approx. (minute)	16-20	16-20	16-20	16-20
Expected Minimum Efficiency (Total theor. plates in 6-ft. column basis p,p'-DDT)	3000	3000	3000	2800

1.5% OV-17/1.95% OV-210

Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202	204	Compound
0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.31	Dimethyl Phthalate
0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	Mevinphos
0.34	0.34	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	Tecnazene
0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.40	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.41	Diethyl Phthalate
0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	2,4-D(ME)
0.42	0.42	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	Hexachlorobenzene
0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.54	0.54	0.54	0.55	α-BHC
0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	CDEC
0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	2,4-D(IPE)
0.54	0.54	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.58	0.58	0.58	0.59	0.59	0.60	0.60	0.60	0.61	Chlordene
0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	Diazinon
0.65	0.65	0.65	0.65	0.66	0.66	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	PCNB
0.66	0.67	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	Lindane
0.76	0.76	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	2,4,5-T(ME)
0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.80	β-BHC
0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	Heptachlor
0.94	0.94	0.93	0.92	0.92	0.91	0.90	0.90	0.89	0.88	0.88	0.67	0.87	0.86	0.85	0.85	0.84	0.83	2,4,5-T(IPE)
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.17	1.16	1.15	1.14	1.13	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.03	1.02	1.01	1.00	0.99	0.98	Dimethoate
1.17	1.16	1.16	1.15	1.14	1.14	1.13	1.12	1.12	1.11	1.10	1.09	1.09	1.08	1.07	1.07	1.06	1.05	Ronnel
1.49	1.48	1.45	1.43	1.41	1.40	1.38	1.36	1.34	1.32	1.31	1.29	1.27	1.25	1.23	1.22	1.20	1.18	Dibutyl Phthalate
1.41	1.40	1.39	1.38	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.24	1.23	1-Hydroxychlordene
1.49	1.49	1.47	1.47	1.46	1.45	1.44	1.44	1.43	1.42	1.42	1.41	1.40	1.39	1.39	1.38	1.37	1.36	Oxychlordane
1.71	1.69	1.67	1.66	1.64	1.62	1.60	1.59	1.57	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.43	1.41	M. Parathion
1.70	1.69	1.68	1.67	1.66	1.65	1.64	1.63	1.62	1.61	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	Heptachlor Epoxide
1.82	1.80	1.78	1.76	1.74	1.72	1.70	1.68	1.66	1.64	1.62	1.60	1.58	1.56	1.54	1.52	1.50	1.48	DCPA
2.07	2.04	2.01	1.98	1.95	1.92	1.89	1.87	1.94	1.81	1.78	1.75	1.72	1.69	1.66	1.63	1.60	1.57	Malathion
1.92	1.91	1.89	1.88	1.85	1.85	1.83	1.81	1.80	1.78	1.77	1.75	1.74	1.72	1.71	1.69	1.68	1.66	Chlordane, Gamma
2.02	2.00	1.99	1.97	1.95	1.93	1.92	1.90	1.88	1.86	1.85	1.83	1.82	1.79	1.78	1.76	1.74	1.73	*Trans*-Nonachlor
2.14	2.12	2.09	2.07	2.05	2.03	2.01	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	1.79	1.77	o,p'-DDE
2.32	2.28	2.25	2.22	2.19	2.16	2.13	2.09	2.06	2.03	2.00	1.97	1.93	1.90	1.87	1.84	1.81	1.78	E. Parathion
2.15	2.13	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	Chlordane, Alpha
2.20	2.18	2.16	2.15	2.13	2.11	2.10	2.08	2.06	2.05	2.03	2.01	2.00	1.98	1.97	1.95	1.93	1.91	Endosulfan I
2.75	2.72	2.68	2.64	2.61	2.58	2.54	2.51	2.47	2.43	2.40	2.37	2.33	2.30	2.27	2.23	2.20	2.17	p,p'-DDE
2.97	2.93	2.88	2.84	2.79	2.75	2.71	2.66	2.62	2.57	2.53	2.49	2.44	2.40	2.35	2.31	2.27	2.22	DDA(ME)
2.80	2.77	2.75	2.72	2.69	2.67	2.64	2.61	2.59	2.56	2.53	2.51	2.48	2.45	2.43	2.40	2.37	2.35	Dieldrin
3.34	3.29	3.25	3.20	3.15	3.11	3.06	3.01	2.97	2.92	2.88	2.83	2.77	2.74	2.59	2.65	2.60	2.56	o,p'-DDD
3.26	3.23	3.19	3.16	3.13	3.09	3.06	3.03	3.00	2.96	2.93	2.90	2.87	2.83	2.80	2.77	2.74	2.70	Chlordecone
3.47	3.43	3.40	3.36	3.33	3.29	3.26	3.22	3.18	3.15	3.12	3.08	3.04	3.01	2.97	2.93	2.90	2.87	Endrin
3.98	3.94	3.88	3.83	3.77	3.71	3.66	3.60	3.54	3.48	3.43	3.38	3.32	3.27	3.21	3.16	3.10	3.04	o,p'-DDT
4.65	4.57	4.49	4.41	4.33	4.26	4.18	4.10	4.02	3.94	3.87	3.79	3.71	3.64	3.61	3.48	3.40	3.32	p,p'-DDD
4.45	4.39	4.34	4.28	4.23	4.17	4.11	4.05	3.99	3.94	3.88	3.82	3.76	3.71	3.65	3.59	3.54	3.48	Endosulfan II
5.57	5.48	5.39	5.29	5.20	5.11	5.01	4.92	4.83	4.74	4.64	4.55	4.46	4.36	4.27	4.18	4.09	4.00	p,p'-DDT
6.1	5.97	5.85	5.73	5.61	5.49	5.36	5.24	5.12	5.00	4.88	4.76	4.64	4.52	4.40	4.28	4.16	4.04	Ethion
6.4	6.2	6.1	5.99	5.88	5.76	5.64	5.52	5.40	5.28	5.16	5.04	4.92	4.80	4.68	4.56	4.44	4.32	Carbophenothion
7.7	7.6	7.5	7.3	7.3	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	5.85	Mirex
10.7	10.5	10.3	10.1	9.9	9.7	9.5	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	7.7	7.5	7.3	Endrin Ketone "153"
13.1	12.7	12.4	12.0	11.6	11.2	10.8	10.4	10.0	9.7	9.3	8.9	8.5	8.1	7.7	7.3	7.0	6.6	Dioctyl Phthalate
12.4	12.1	11.8	11.6	11.3	11.0	10.7	10.4	10.1	9.8	9.5	9.3	9.0	8.7	8.4	8.1	7.8	7.5	Methoxychlor
16.9	16.5	16.1	15.7	15.3	14.9	14.5	14.1	13.7	13.3	12.9	12.5	12.1	11.7	11.3	10.9	10.5	10.2	Tetradifon
22.1	21.5	20.9	20.3	19.6	19.0	18.4	17.7	17.1	16.5	15.8	15.2	14.6	14.0	13.4	12.7	12.1	11.5	Diphenyl Phthalate
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to aldrin, of 49 compounds at temperatures from 170 to 204°C; support of Gas Chrom Q, 100/120 mesh; electron capture detector; tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicated optimum column operating temperature with carrier flow at 60 ml per minute.

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Table 2(b)

4%SE-30/6%OV-210

Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202	204	Compound
0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.30	0.30	0.30	Dimethyl Phthalate
0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.33	Mevinphos
0.34	0.34	0.34	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	Tecnazene
0.39	0.39	0.39	0.39	0.40	0.40	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	Diethyl Phthalate
0.39	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	0.44	0.44	0.45	2,4-D(ME)
0.39	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.43	0.43	0.43	0.44	0.44	0.45	0.45	0.45	0.46	0.46	Hexachlorobenzene
0.42	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.50	α-BHC
0.44	0.44	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	CDEC
0.54	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	2,4-D(IPE)
0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	Lindane
0.54	0.54	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.58	0.58	0.58	0.59	0.59	0.60	0.60	0.60	0.61	Chlordene
0.57	0.57	0.57	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	β-BHC
0.60	0.60	0.60	0.60	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.58	0.58	0.58	0.58	0.58	0.58	0.58	Diazinon
0.59	0.59	0.60	0.60	0.60	0.61	0.61	0.61	0.62	0.62	0.62	0.63	0.63	0.63	0.64	0.64	0.64	0.65	PCNB
0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	0.64	2,4,5-T(ME)
0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.83	0.83	0.83	Heptachlor
0.89	0.89	0.88	0.88	0.87	0.87	0.87	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.83	0.83	0.83	0.82	2,4,5-T(IPE)
0.96	0.95	0.94	0.94	0.93	0.93	0.92	0.91	0.91	0.90	0.89	0.89	0.88	0.87	0.87	0.86	0.85	0.85	Dimethoate
1.01	1.00	1.00	0.99	0.98	0.98	0.97	0.96	0.96	0.95	0.94	0.94	0.93	0.92	0.92	0.91	0.90	0.90	Ronnel
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.02	1-Hydroxychlordene
1.41	1.39	1.37	1.36	1.34	1.33	1.31	1.30	1.28	1.27	1.25	1.24	1.22	1.21	1.19	1.18	1.16	1.14	Dibutyl Phthalate
1.43	1.42	1.42	1.41	1.40	1.40	1.39	1.39	1.38	1.37	1.37	1.36	1.36	1.35	1.34	1.34	1.33	1.33	Oxychlordane
1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	1.35	1.34	1.33	1.32	M. Parathion
1.53	1.53	1.52	1.51	1.50	1.50	1.49	1.48	1.48	1.47	1.46	1.46	1.45	1.44	1.44	1.43	1.42	1.42	Heptachlor Epoxide
1.64	1.63	1.61	1.60	1.59	1.57	1.56	1.54	1.53	1.52	1.51	1.49	1.48	1.47	1.45	1.44	1.43	1.41	D C P A
1.70	1.68	1.66	1.64	1.63	1.61	1.59	1.57	1.55	1.53	1.51	1.49	1.48	1.46	1.44	1.42	1.40	1.38	Malathion
1.67	1.66	1.65	1.63	1.62	1.60	1.59	1.57	1.56	1.55	1.53	1.52	1.50	1.49	1.47	1.46	1.45	1.43	o,p'-DDE
1.65	1.64	1.63	1.62	1.61	1.61	1.60	1.59	1.58	1.57	1.56	1.55	1.54	1.54	1.53	1.52	1.52	1.50	Chlordane, Gamma
1.84	1.83	1.82	1.80	1.79	1.78	1.77	1.76	1.75	1.74	1.73	1.71	1.70	1.69	1.68	1.67	1.66	1.65	Chlordane, Alpha
1.86	1.85	1.83	1.82	1.81	1.80	1.78	1.77	1.76	1.74	1.73	1.72	1.71	1.69	1.68	1.67	1.66	1.64	*Trans*-Nonachlor
2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.94	1.91	1.89	1.87	1.85	1.83	1.80	1.78	1.76	1.74	1.72	E. Parathion
1.99	1.98	1.97	1.95	1.94	1.93	1.91	1.90	1.89	1.87	1.86	1.85	1.83	1.82	1.80	1.79	1.78	1.76	Endosulfan I
2.16	2.14	2.11	2.09	2.07	2.05	2.02	2.00	1.98	1.95	1.93	1.91	1.89	1.86	1.84	1.82	1.80	1.77	p,p'-DDE
2.27	2.25	2.22	2.19	2.16	2.13	2.10	2.07	2.04	2.01	1.98	1.96	1.93	1.90	1.87	1.84	1.81	1.78	DDA(ME)
2.34	2.31	2.29	2.27	2.24	2.22	2.19	2.17	2.15	2.12	2.10	2.07	2.05	2.03	2.00	1.98	1.96	1.93	o,p'-DDD
2.43	2.41	2.39	2.37	2.35	2.33	2.31	2.29	2.27	2.25	2.22	2.20	2.18	2.16	2.14	2.12	2.10	2.08	Dieldrin
3.02	2.97	2.93	2.88	2.84	2.80	2.76	2.73	2.68	2.64	2.60	2.56	2.52	2.47	2.43	2.39	2.35	2.31	o,p'-DDT
2.76	2.73	2.71	2.69	2.67	2.64	2.62	2.60	2.58	2.55	2.53	2.51	2.49	2.46	2.44	2.42	2.40	2.37	Endrin
3.22	3.17	3.13	3.08	3.04	2.98	2.94	2.90	2.86	2.82	2.77	2.73	2.68	2.64	2.59	2.55	2.51	2.46	p,p'-DDD
2.97	2.94	2.91	2.89	2.86	2.83	2.80	2.78	2.75	2.72	2.69	2.67	2.64	2.61	2.59	2.56	2.53	2.50	Chlordecone
3.19	3.16	3.13	3.10	3.07	3.04	3.00	2.97	2.94	2.91	2.88	2.85	2.81	2.78	2.75	2.72	2.69	2.66	Endosulfan II
4.08	4.02	3.96	3.89	3.81	3.76	3.68	3.60	3.53	3.47	3.40	3.32	3.27	3.20	3.13	3.05	2.98	2.90	Ethion
4.04	3.98	3.92	3.86	3.80	3.73	3.67	3.61	3.54	3.48	3.43	3.36	3.30	3.24	3.18	3.12	3.05	2.98	p,p'-DDT
4.08	4.02	3.98	3.90	3.83	3.78	3.72	3.56	3.59	3.52	3.47	3.40	3.34	3.28	3.22	3.16	3.10	3.03	Carbophenothion
6.7	6.5	6.4	6.3	6.1	5.98	5.84	5.70	5.57	5.42	5.29	5.16	5.01	4.88	4.73	4.60	4.46	4.32	Methoxychlor
6.1	6.0	5.96	5.87	5.78	5.68	5.60	5.52	5.43	5.33	5.24	5.15	5.06	4.97	4.88	4.79	4.70	4.62	Mirex
7.3	7.2	7.1	7.0	6.9	6.8	6.7	6.5	6.4	6.3	6.2	6.1	5.98	5.84	5.75	5.64	5.53	5.42	Endrin Ketone"153"
11.0	10.8	10.5	10.2	9.9	9.6	9.3	9.0	8.7	8.4	8.1	7.8	7.5	7.2	6.9	6.6	6.4	6.1	Dioctyl Phthalate
12.2	11.9	11.6	11.3	11.0	10.7	10.4	10.1	9.8	9.5	9.2	8.9	8.6	8.3	8.0	7.7	7.4	7.1	Diphenyl Phthalate
11.6	11.3	11.1	10.8	10.6	10.3	10.1	9.8	9.6	9.3	9.0	8.8	8.5	8.3	8.0	7.8	7.5	7.3	Tetradifon
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to aldrin, of 49 compounds at temperatures from 170 to 204°C; support of Gas Chrom Q, 80/100 mesh; electron capture detector; tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicated optimum column operating temperature with carrier flow at 70 ml per minute.

Table 2(c)

5 % OV-210

Column Temperature, °C.

170		174		178	↓	182		186		190		194		198		202	204	Compound
0.43	0.43	0.44	0.45	0.45	0.46	0.46	0.47	0.48	0.48	0.49	0.49	0.50	0.51	0.51	0.52	0.52	0.53	Hexachlorobenzene
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	Dimethyl Phthalate
0.52	0.53	0.53	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.58	0.58	0.59	0.59	0.60	0.60	Tecnazene
0.58	0.59	0.59	0.60	0.60	0.61	0.61	0.62	0.62	0.63	0.63	0.64	0.64	0.65	0.65	0.66	0.67	0.67	Chlordene
0.58	0.58	0.59	0.60	0.61	0.62	0.62	0.63	0.64	0.65	0.66	0.66	0.67	0.68	0.69	0.70	0.70	0.71	α-BHC
0.65	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.69	0.69	0.69	0.70	0.70	0.70	0.70	CDEC
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	Mevinphos
0.73	0.73	0.72	0.72	0.72	0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.70	0.70	0.70	0.70	0.70	0.69	Diethyl Phthalate
0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.72	0.72	0.72	0.71	0.71	0.71	0.71	0.70	Diazinon
0.78	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	0.82	Lindane
0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.84	0.83	0.83	0.83	0.83	0.83	0.82	0.82	0.82	0.82	0.82	2,4-D(IPE)
0.83	0.83	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	0.85	PCNB
0.86	0.86	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.88	0.88	0.88	0.88	0.88	0.88	Heptachlor
0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.91	0.91	β-BHC
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.41	1.39	1.38	1.37	1.35	1.34	1.33	1.32	1.30	1.29	1.28	1.26	1.25	1.24	1.22	1.21	1.20	1.19	Ronnel
1.44	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1-Hydroxychlordene
1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	1.51	1.50	1.49	1.48	1.47	1.45	1.44	1.43	1.42	1.41	Oxychlordane
1.66	1.64	1.63	1.61	1.60	1.58	1.56	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.44	1.42	1.40	1.39	o,p'-DDE
1.88	1.87	1.85	1.83	1.81	1.80	1.78	1.76	1.74	1.73	1.71	1.69	1.68	1.66	1.64	1.62	1.61	1.59	*Trans*-Nonachlor
1.88	1.87	1.85	1.83	1.82	1.80	1.78	1.77	1.75	1.73	1.72	1.70	1.68	1.67	1.65	1.63	1.62	1.60	Chlordane, Gamma
1.96	1.94	1.92	1.91	1.89	1.87	1.85	1.84	1.83	1.80	1.79	1.77	1.75	1.74	1.72	1.70	1.68	1.67	Heptachlor Epoxide
2.05	2.03	2.02	2.00	1.98	1.96	1.94	1.92	1.90	1.89	1.87	1.85	1.83	1.81	1.79	1.77	1.75	1.74	Chlordane, Alpha
2.25	2.21	2.17	2.13	2.10	2.06	2.02	1.99	1.95	1.91	1.87	1.84	1.80	1.76	1.72	1.69	1.65	1.61	Dibutyl Phthalate
2.21	2.18	2.16	2.13	2.10	2.09	2.04	2.01	1.98	1.95	1.92	1.89	1.87	1.84	1.81	1.78	1.75	1.72	Dimethoate
2.21	2.19	2.16	2.13	2.11	2.09	2.05	2.02	2.00	1.97	1.94	1.92	1.89	1.86	1.84	1.81	1.78	1.75	p,p'-DDE
2.54	2.52	2.49	2.46	2.44	2.41	2.38	2.35	2.33	2.30	2.27	2.25	2.22	2.19	2.17	2.14	2.11	2.08	Endosulfan I
2.60	2.58	2.55	2.53	2.50	2.48	2.46	2.43	2.41	2.38	2.36	2.34	2.31	2.29	2.26	2.24	2.22	2.19	o,p'-DDD
2.69	2.65	2.61	2.57	2.55	2.49	2.45	2.41	2.37	2.33	2.29	2.25	2.21	2.17	2.13	2.09	2.05	2.01	DCPA
2.83	2.79	2.76	2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	2.45	2.42	2.38	2.35	2.32	2.28	2.25	Chlordecone
2.97	2.92	2.86	2.81	2.75	2.70	2.65	2.59	2.54	2.48	2.43	2.38	2.32	2.27	2.21	2.16	2.11	2.05	o,p'-DDT
3.00	2.95	2.89	2.84	2.79	2.73	2.68	2.63	2.58	2.52	2.47	2.42	2.36	2.31	2.26	2.21	2.15	2.10	Malathion
2.95	2.91	2.87	2.82	2.78	2.74	2.70	2.66	2.61	2.57	2.53	2.49	2.45	2.40	2.36	2.32	2.28	2.24	M. Parathion
3.08	3.05	3.01	2.98	2.94	2.91	2.87	2.84	2.80	2.77	2.73	2.70	2.66	2.63	2.59	2.56	2.52	2.49	Dieldrin
3.71	3.66	3.61	3.56	3.51	3.46	3.41	3.36	3.31	3.26	3.21	3.12	3.11	3.06	3.01	2.96	2.91	2.86	Endrin
4.01	3.94	3.88	3.81	3.74	3.67	3.60	3.53	3.46	3.39	3.32	3.25	3.19	3.12	3.05	2.98	2.91	2.84	p,p'-DDD
4.45	4.31	4.17	4.04	3.90	3.76	3.62	3.49	3.35	3.21	3.08	2.94	2.80	2.67	2.53	2.39	2.25	2.12	E. Parathion
4.15	4.09	4.03	3.98	3.92	3.86	3.80	3.74	3.69	3.63	3.57	3.51	3.45	3.40	3.34	3.28	3.22	3.16	Mirex
4.38	4.31	4.23	4.16	4.08	4.01	3.93	3.85	3.78	3.70	3.63	3.55	3.47	3.40	3.32	3.25	3.17	3.09	p,p'-DDT
4.78	4.70	4.63	4.55	4.48	4.40	4.33	4.25	4.18	4.11	4.03	3.96	3.88	3.81	3.73	3.66	3.58	3.50	Endosulfan II
5.28	5.17	5.06	4.95	4.84	4.73	4.62	4.51	4.40	4.28	4.17	4.06	3.95	3.84	3.73	3.62	3.51	3.40	Carbophenothion
5.90	5.77	5.63	5.50	5.36	5.23	5.09	4.96	4.82	4.69	4.55	4.42	4.28	4.15	4.01	3.89	3.74	3.61	Ethion
7.3	7.1	6.9	6.7	6.55	6.4	6.2	6.0	5.84	5.66	5.49	5.31	5.13	4.95	4.77	4.60	4.42	4.24	Methoxychlor
13.6	13.1	12.7	12.3	11.9	11.4	11.0	10.6	10.2	9.7	9.3	8.9	8.5	8.0	7.6	7.2	6.8	6.4	Dioctyl Phthalate
12.9	12.6	12.3	12.0	11.8	11.5	11.2	10.9	10.6	10.3	10.1	9.8	9.5	9.2	8.9	8.6	8.4	8.1	Endrin Ketone "153"
20.0	19.4	18.9	18.3	17.8	17.3	16.7	16.2	15.6	15.1	14.6	14.0	13.5	13.0	12.4	11.9	11.3	10.8	Tetradifon
21.0	20.4	19.7	19.1	18.5	17.8	17.2	16.5	15.9	15.2	14.6	13.9	13.3	12.6	12.0	11.4	10.7	10.1	Diphenyl Phthalate

170 174 178 182 186 190 194 198 202 204

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Retention ratios, relative to aldrin, of 47 compounds at temperatures from 170 to 204°C; support of gas Chrom Q, 80/100 mesh; electron capture detector; ^{63}Ni source; all absolute retentions measured from injection point. Arrow indicated optimum column operating temperature with carrier flow at 50 ml per min.

Table 2(d)

10% DC-200
Column Temperature, °C

170		174		178		182		186		190		194		198	↓	202	204	
0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.20	**Mevinphos**
0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.33	2,4-D(ME)
0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	**Phorate**
0.35	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.39	0.39	0.40	0.40	0.41	0.41	0.42	0.42	0.42	0.43	α-BHC
0.35	0.35	0.36	0.36	0.37	0.37	0.37	0.38	0.38	0.39	0.39	0.39	0.40	0.40	0.41	0.41	0.41	0.42	CDEC
0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	2,4-D(IPE)
0.39	0.39	0.39	0.39	0.40	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.42	Simazine
0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.43	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.44	Atrazine
0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	Diazinon
0.43	0.43	0.44	0.44	0.45	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	Lindane
0.48	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.50	2,4,5-T(ME)
0.38	0.38	0.39	0.39	0.40	0.40	0.41	0.41	0.42	0.42	0.43	0.43	0.44	0.44	0.45	0.45	0.46	0.46	β BHC
0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	2,4-D(BE)I
0.45	0.45	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.51	0.51	0.51	0.52	δ-BHC
0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.82	Heptachlor
0.67	0.67	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	2,4,5-T(IPE)
0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69	0.69	0.69	0.69	2,4-D(BE)II
0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.53	0.53	Dichlone
0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
0.82	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.78	0.78	0.78	0.78	Ronnel
1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99	0.98	1-Hydroxychlordene
0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	M. Parathion
1.29	1.29	1.28	1.28	1.27	1.27	1.26	1.26	1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.20	1.20	Heptachlor Epoxide
0.77	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.82	Malathion
1.10	1.09	1.09	1.08	1.08	1.07	1.06	1.06	1.05	1.05	1.04	1.03	1.03	1.02	1.02	1.01	1.01	1.00	**D C P A**
1.29	1.28	1.27	1.27	1.26	1.25	1.24	1.24	1.23	1.22	1.22	1.21	1.20	1.20	1.19	1.18	1.17	1.17	Dyrene
1.65	1.64	1.62	1.61	1.59	1.58	1.57	1.55	1.54	1.53	1.51	1.50	1.48	1.47	1.46	1.44	1.43	1.41	o,p'-DDE
1.43	1.42	1.41	1.40	1.40	1.39	1.38	1.37	1.36	1.36	1.35	1.34	1.33	1.33	1.32	1.31	1.30	1.30	**Chlorbenside**
1.03	1.02	1.02	1.01	1.00	1.00	0.99	0.99	0.98	0.98	0.97	0.96	0.96	0.95	0.95	0.94	0.93	0.93	E. Parathion
1.64	1.63	1.62	1.61	1.61	1.60	1.59	1.58	1.58	1.57	1.56	1.55	1.54	1.54	1.53	1.52	1.51	1.50	**Endosulfan I**
2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.83	1.81	1.79	1.77	1.75	1.73	p,p'-DDE
1.81	1.79	1.77	1.75	1.73	1.71	1.69	1.67	1.65	1.63	1.61	1.60	1.58	1.56	1.54	1.52	1.50	1.48	DDA(ME)
1.21	1.21	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.14	1.13	Captan
1.30	1.29	1.29	1.28	1.27	1.27	1.26	1.26	1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.20	1.20	Folpet
1.99	1.98	1.96	1.95	1.93	1.92	1.90	1.89	1.87	1.86	1.84	1.83	1.81	1.80	1.79	1.77	1.76	1.74	Dieldrin
2.60	2.56	2.53	2.49	2.45	2.41	2.38	2.34	2.30	2.27	2.23	2.19	2.15	2.11	2.08	2.04	2.00	1.97	Perthane
2.13	2.11	2.08	2.06	2.04	2.01	1.99	1.97	1.94	1.92	1.90	1.87	1.85	1.83	1.80	1.78	1.76	1.73	o,p'-DDD
2.90	2.86	2.83	2.79	2.76	2.72	2.68	2.65	2.61	2.58	2.54	2.50	2.47	2.43	2.40	2.36	2.32	2.29	o,p'-DDT
2.22	2.20	2.19	2.17	2.16	2.14	2.13	2.11	2.09	2.08	2.06	2.05	2.03	2.01	2.00	1.98	1.97	1.95	Endrin
3.07	3.03	3.00	2.96	2.93	2.90	2.86	2.83	2.79	2.76	2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	**Chlordecone**
2.72	2.69	2.66	2.62	2.59	2.56	2.52	2.49	2.46	2.42	2.39	2.35	2.32	2.29	2.25	2.22	2.19	2.15	p,p'-DDD
2.29	2.27	2.25	2.24	2.22	2.20	2.18	2.17	2.15	2.13	2.11	2.10	2.08	2.06	2.05	2.03	2.01	2.00	**Endosulfan II**
3.04	3.00	2.95	2.91	2.86	2.81	2.77	2.72	2.68	2.63	2.58	2.54	2.49	2.44	2.40	2.35	2.31	2.26	Ethion
3.72	3.67	3.62	3.57	3.51	3.46	3.40	3.35	3.29	3.24	3.18	3.13	3.08	3.02	2.97	2.92	2.86	2.81	p,p'-DDT
3.42	3.37	3.32	3.26	3.21	3.16	3.11	3.06	3.01	2.96	2.90	2.85	2.80	2.75	2.70	2.65	2.60	2.55	**Carbophenothion**
3.23	3.19	3.15	3.11	3.06	3.02	2.98	2.93	2.89	2.85	2.80	2.76	2.72	2.67	2.63	2.58	2.54	2.50	Dilan I
6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	5.90	5.80	5.69	5.59	5.48	5.38	5.28	5.17	Mirex
6.1	6.0	5.89	5.77	5.66	5.54	5.42	5.30	5.18	5.06	4.93	4.82	4.69	4.58	4.45	4.33	4.22	4.10	Methoxychlor
4.07	4.02	3.96	3.89	3.83	3.77	3.70	3.64	3.58	3.52	3.45	3.39	3.33	3.27	3.20	3.14	3.08	3.02	Dilan II
6.4	6.3	6.2	6.1	5.97	5.85	5.73	5.62	5.50	5.38	5.27	5.15	5.03	4.92	4.80	4.68	4.57	4.45	**Tetradifon**
6.4	6.3	6.2	6.0	5.92	5.81	5.69	5.58	5.46	5.34	5.23	5.12	5.00	4.88	4.77	4.66	4.54	4.42	**Azinphosmethyl**
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to aldrin, of 48 pesticides on a column of 10% DC-200 at temperatures from 170 to 204°C; support of Chromosorb W,H.P , 80/100 mesh; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 120 ml per minute.

Table 2(e)

5%DC-200/7.5%QF-1
Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202	204	
0.29	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.31	**Mevinphos**
0.40	0.40	0.40	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.42	0.42	2,4-D(ME)
0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.45	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.46	0.46	**Phorate**
0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.48	0.49	α BHC
0.45	0.46	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.50	CDEC
0.54	0.54	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.55	0.55	0.55	0.55	0.55	2,4-D(IPE)
0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	Simazine
0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.57	0.57	0.57	Atrazine
0.59	0.59	0.59	0.59	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.57	0.57	0.57	0.57	0.57	0.57	0.57	Diazinon
0.55	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.61	Lindane
0.67	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	2,4,5-T(ME)
0.57	0.58	0.58	0.58	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.60	β BHC
0.83	0.83	0.83	0.82	0.82	0.82	0.81	0.81	0.81	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.77	2,4-D(BE)I
0.65	0.65	0.66	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.70	δ-BHC
0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82	0.82	0.82	Heptachlor
0.90	0.89	0.89	0.89	0.88	0.88	0.87	0.87	0.86	0.86	0.85	0.85	0.84	0.84	0.83	0.83	0.82	0.82	2,4,5-T(IPE)
0.98	0.97	0.96	0.95	0.95	0.94	0.93	0.92	0.91	0.91	0.90	0.89	0.89	0.88	0.87	0.86	0.85	0.85	2,4-D(BE)II
0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	Dichlone
0.95	0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.90	0.89	0.89	0.89	0.88	0.88	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.02	1.01	1.01	1.01	1.00	1.00	1.00	0.99	0.99	0.99	0.98	0.98	0.98	0.96	0.96	0.95	0.96	0.94	Ronnel
1.27	1.27	1.25	1.24	1.23	1.22	1.21	1.20	1.19	1.18	1.17	1.16	1.15	1.14	1.13	1.12	1.11	1.10	1 Hydroxychlordene
1.54	1.53	1.51	1.51	1.49	1.49	1.47	1.46	1.45	1.44	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	M. Parathion
1.54	1.53	1.53	1.52	1.51	1.51	1.50	1.49	1.49	1.48	1.47	1.47	1.46	1.45	1.45	1.44	1.43	1.43	Heptachlor Epoxide
1.73	1.71	1.69	1.67	1.65	1.63	1.61	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.46	1.43	1.41	1.39	Malathion
1.70	1.68	1.67	1.65	1.63	1.62	1.60	1.59	1.57	1.55	1.54	1.52	1.51	1.49	1.48	1.46	1.44	1.43	**D C P A**
1.54	1.53	1.51	1.50	1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.41	1.40	1.39	1.38	1.37	1.36	1.35	Dyrene
1.66	1.65	1.63	1.62	1.61	1.59	1.58	1.57	1.56	1.54	1.53	1.51	1.50	1.49	1.47	1.46	1.45	1.43	o,p' DDE
1.62	1.61	1.60	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	1.51	1.50	1.49	1.48	1.47	1.46	1.45	**Chlorbenside**
2.14	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	1.95	1.93	1.91	1.88	1.86	1.84	1.82	1.80	1.78	E. Parathion
2.00	1.99	1.97	1.96	1.95	1.94	1.93	1.91	1.90	1.89	1.88	1.87	1.86	1.85	1.83	1.82	1.81	1.80	**Endosulfan I**
2.15	2.13	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	1.95	1.93	1.90	1.88	1.86	1.84	1.82	1.80	p,p'-DDE
2.28	2.25	2.22	2.19	2.16	2.13	2.10	2.07	2.04	2.01	1.99	1.96	1.93	1.90	1.87	1.84	1.81	1.78	DDA(ME)
2.35	2.33	2.31	2.28	2.26	2.23	2.21	2.19	2.17	2.14	2.12	2.09	2.07	2.05	2.02	2.00	1.98	1.95	Captan
2.24	2.23	2.21	2.19	2.17	2.15	2.13	2.12	2.10	2.08	2.06	2.04	2.03	2.01	1.99	1.97	1.95	1.93	Folpet
2.40	2.38	2.37	2.35	2.33	2.32	2.30	2.29	2.27	2.25	2.24	2.22	2.21	2.19	2.18	2.16	2.15	2.13	Dieldrin
2.42	2.39	2.36	2.33	2.30	2.27	2.24	2.21	2.18	2.15	2.12	2.09	2.05	2.02	1.99	1.96	1.93	1.90	Perthane
2.37	2.35	2.33	2.30	2.28	2.25	2.23	2.21	2.18	2.16	2.13	2.11	2.08	2.06	2.03	2.01	1.99	1.96	o,p'-DDD
2.91	2.87	2.84	2.81	2.78	2.75	2.72	2.69	2.65	2.62	2.59	2.56	2.53	2.49	2.46	2.43	2.40	2.37	o,p'-DDT
2.81	2.79	2.76	2.74	2.72	2.69	2.67	2.65	2.62	2.60	2.58	2.55	2.53	2.51	2.48	2.46	2.44	2.41	Endrin
2.96	2.93	2.91	2.89	2.86	2.84	2.81	2.79	2.76	2.74	2.71	2.69	2.66	2.64	2.61	2.59	2.57	2.54	**Chlordecone**
3.22	3.18	3.14	3.10	3.06	3.02	2.98	2.94	2.90	2.86	2.82	2.78	2.74	2.70	2.66	2.62	2.58	2.54	p,p'-DDD
3.22	3.19	3.16	3.13	3.11	3.07	3.05	3.02	2.99	2.96	2.93	2.90	2.87	2.84	2.81	2.78	2.75	2.72	**Endosulfan II**
4.10	4.04	3.98	3.91	3.85	3.79	3.73	3.66	3.60	3.54	3.47	3.41	3.35	3.29	3.22	3.16	3.10	3.03	Ethion
4.07	4.00	3.93	3.86	3.79	3.72	3.65	3.58	3.51	3.44	3.37	3.30	3.23	3.16	3.09	3.02	2.95	2.88	p,p'-DDT
4.12	4.06	4.00	3.94	3.88	3.82	3.76	3.70	3.63	3.57	3.50	3.44	3.38	3.32	3.26	3.20	3.14	3.08	**Carbophenothion**
6.3	6.2	6.1	5.98	5.87	5.76	5.65	5.53	5.42	5.31	5.20	5.10	5.00	4.88	4.78	4.68	4.56	4.46	Dilan I
5.98	5.91	5.83	5.76	5.68	5.61	5.53	5.46	5.38	5.30	5.22	5.15	5.08	5.00	4.92	4.85	4.78	4.70	Mirex
6.5	6.5	6.4	6.2	6.1	5.97	5.83	5.71	5.56	5.43	5.30	5.18	5.03	4.88	4.78	4.64	4.50	4.38	Methoxychlor
7.5	7.3	7.2	7.0	6.9	6.8	6.6	6.5	6.4	6.2	6.1	5.94	5.80	5.67	5.53	5.40	5.26	5.11	Dilan II
11.6	11.4	11.2	10.9	10.7	10.4	10.2	10.0	9.7	9.5	9.2	9.0	8.8	8.5	8.3	8.0	7.8	7.6	**Tetradifon**
12.5	12.2	12.0	11.7	11.4	11.2	10.9	10.6	10.4	10.1	9.9	9.6	9.3	9.1	8.8	8.5	8.3	8.0	**Azinphosmethyl**
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to aldrin, of 48 pesticides on a column of 5% DC-200/7.5%QF-1 at temperatures from 170 to 204°C; support of Chromosorb W,H.P., 80/100 mesh; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 120 ml per minute.

Table 2(f)

1.6%OV-17/6.4%OV-210

Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202		
0.36	0.37	0.37	0.37	0.37	0.37	0.3[illegible]	0.38	0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.39	Mevinphos
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.51	2,4-D(ME)
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.54	Phorate
0.49	0.50	0.50	0.51	0.52	0.52	0.52	0.53	0.53	0.54	0.54	0.55	0.55	0.56	0.57	0.57	0.58	0.58	α-BHC
0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.57	0.58	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.59	0.60	CDEC
0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	2,4-D(IPE)
0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	Simazine
0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	Atrazine
0.71	0.70	0.70	0.69	0.69	0.69	0.68	0.68	0.67	0.67	0.66	0.66	0.65	0.65	0.64	0.64	0.64	0.63	Diazinon
0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.71	0.71	0.71	0.71	Lindane
0.84	0.84	0.83	0.83	0.82	0.82	0.82	0.81	0.81	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.77	2,4,5-T(ME)
0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	β-BHC
0.96	0.96	0.95	0.95	0.94	0.93	0.93	0.92	0.91	0.91	0.90	0.90	0.89	0.88	0.88	0.87	0.86	0.86	2,4-D(BE)I
0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	δ-BHC
0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	Heptachlor
1.02	1.02	1.01	1.01	1.00	1.00	0.99	0.99	0.98	0.98	0.98	0.97	0.96	0.96	0.96	0.95	0.95	0.94	2,4,5-T(IPE)
1.13	1.12	1.12	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.04	1.04	1.03	1.02	1.01	1.00	0.99	0.98	2,4-D(BE)II
1.14	1.14	1.14	1.14	1.14	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.12	1.12	1.12	1.12	1.12	1.12	Dichlone
1.30	1.29	1.29	1.28	1.28	1.27	1.26	1.26	1.25	1.25	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	Dimethoate
1.00																	→	Aldrin (REFERENCE)
1.24	1.23	1.22	1.22	1.21	1.20	1.20	1.19	1.18	1.17	1.17	1.16	1.15	1.15	1.14	1.13	1.13	1.12	Ronnel
1.43	1.42	1.41	1.41	1.40	1.40	1.39	1.38	1.38	1.37	1.36	1.36	1.35	1.34	1.34	1.33	1.33	1.32	1-Hydroxychlordene
2.02	2.01	1.99	1.98	1.96	1.95	1.93	1.92	1.90	1.88	1.87	1.85	1.84	1.82	1.81	1.79	1.78	1.76	M. Parathion
1.81	1.79	1.78	1.77	1.76	1.75	1.73	1.72	1.71	1.70	1.69	1.68	1.66	1.65	1.64	1.63	1.62	1.60	Heptachlor Epoxide
2.37	2.33	2.30	2.26	2.22	2.18	2.15	2.11	2.07	2.04	2.00	1.96	1.93	1.89	1.86	1.82	1.78	1.74	Malathion
2.06	2.04	2.02	1.99	1.97	1.95	1.93	1.91	1.89	1.86	1.84	1.82	1.80	1.77	1.75	1.73	1.71	1.69	DCPA
2.14	2.12	2.09	2.07	2.04	2.02	2.00	1.97	1.95	1.92	1.90	1.87	1.85	1.83	1.80	1.78	1.76	1.73	Dyrene
2.04	2.02	2.00	1.98	1.95	1.93	1.91	1.89	1.87	1.85	1.83	1.80	1.78	1.76	1.74	1.72	1.70	1.68	o,p'-DDE
2.18	2.16	2.14	2.11	2.09	2.07	2.04	2.02	2.00	1.97	1.95	1.93	1.90	1.88	1.86	1.84	1.81	1.79	Chlorbenside
2.80	2.76	2.73	2.69	2.66	2.62	2.59	2.55	2.52	2.48	2.44	2.41	2.37	2.34	2.30	2.27	2.23	2.20	E. Parathion
2.33	2.31	2.29	2.27	2.25	2.23	2.21	2.19	2.18	2.16	2.14	2.12	2.10	2.08	2.07	2.05	2.03	2.01	Endosulfan I
2.67	2.64	2.60	2.57	2.53	2.49	2.46	2.42	2.39	2.35	2.32	2.28	2.24	2.21	2.17	2.14	2.10	2.06	p,p'-DDE
3.07	3.02	2.97	2.92	2.87	2.82	2.78	2.73	2.68	2.63	2.58	2.54	2.49	2.44	2.39	2.34	2.29	2.24	DDA(ME)
3.58	3.54	3.49	3.44	3.40	3.35	3.30	3.26	3.21	3.17	3.12	3.07	3.03	2.98	2.94	2.89	2.84	2.80	Captan
3.51	3.46	3.42	3.37	3.33	3.28	3.24	3.20	3.15	3.10	3.06	3.02	2.97	2.93	2.88	2.84	2.79	2.75	Folpet
2.94	2.92	2.89	2.86	2.83	2.80	2.77	2.74	2.71	2.68	2.65	2.62	2.60	2.57	2.54	2.51	2.48	2.45	Dieldrin
3.24	3.18	3.12	3.07	3.01	2.95	2.90	2.84	2.78	2.72	2.66	2.61	2.55	2.49	2.44	2.38	2.32	2.26	Perthane
3.26	3.20	3.16	3.10	3.05	3.00	2.96	2.90	2.86	2.80	2.76	2.71	2.66	2.61	2.56	2.51	2.46	2.40	o,p'-DDD
3.81	3.75	3.70	3.64	3.58	3.52	3.46	3.40	3.35	3.28	3.23	3.17	3.11	3.05	3.00	2.94	2.88	2.82	o,p'-DDT
3.68	3.64	3.59	3.54	3.50	3.45	3.40	3.36	3.32	3.27	3.22	3.18	3.13	3.09	3.04	2.99	2.95	2.90	Endrin
3.18	3.15	3.12	3.09	3.06	3.03	3.00	2.97	2.94	2.91	2.88	2.85	2.82	2.80	2.77	2.74	2.71	2.68	Chlordecone
4.63	4.55	4.46	4.38	4.30	4.21	4.13	4.05	3.96	3.88	3.80	3.72	3.63	3.55	3.46	3.38	3.30	3.22	p,p'-TDE
4.60	4.54	4.48	4.42	4.35	4.30	4.24	4.17	4.12	4.05	4.00	3.94	3.88	3.82	3.75	3.69	3.63	3.57	Endosulfan II
6.2	6.0	5.92	5.79	5.67	5.54	5.42	5.29	5.16	5.04	4.92	4.79	4.67	4.54	4.42	4.29	4.17	4.04	Ethion
5.46	5.36	5.26	5.16	5.06	4.96	4.86	4.76	4.66	4.56	4.46	4.36	4.26	4.16	4.06	3.96	3.86	3.76	p,p'-DDT
6.35	6.2	6.1	5.96	5.83	5.69	5.56	5.43	5.30	5.16	5.03	4.90	4.77	4.63	4.50	4.37	4.24	4.10	Carbophenothion
9.1	8.9	8.7	8.5	8.3	8.2	8.0	7.8	7.6	7.4	7.2	7.0	6.8	6.7	6.5	6.3	6.1	5.90	Dilan I
7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	5.97	5.87	5.77	5.67	5.57	5.48	5.38	5.28	Mirex
10.9	10.6	10.4	10.2	10.0	9.7	9.4	9.2	9.0	8.8	8.5	8.3	8.0	7.8	7.6	7.3	7.1	6.8	Methoxychlor
10.5	10.3	10.0	9.8	9.6	9.4	9.1	8.9	8.7	8.4	8.2	8.0	7.8	7.5	7.3	7.1	6.8	6.6	Dilan II
17.5	17.2	16.8	16.4	16.0	15.7	15.3	14.9	14.5	14.1	13.8	13.4	13.0	12.6	12.3	11.9	11.5	11.1	Tetradifon
24.5	23.8	23.2	22.5	21.9	21.3	20.7	20.0	19.4	18.8	18.2	17.6	17.0	16.3	15.7	15.1	14.5	13.8	Azinphosmethyl

Retention ratios, relative to aldrin, of 49 pesticides on a column of 1.6%OV-17/6.4%OV-210 at temperatures from 170 to 204°C; support of Chromosorb W,H.P., 80/100 mesh; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml per minute.

1.5% OV-17/1.95% QF-1

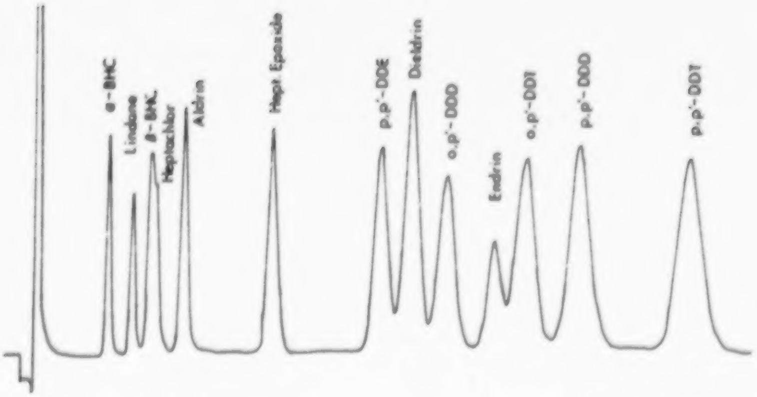


Figure 1

4% SE-30/6% OV-210

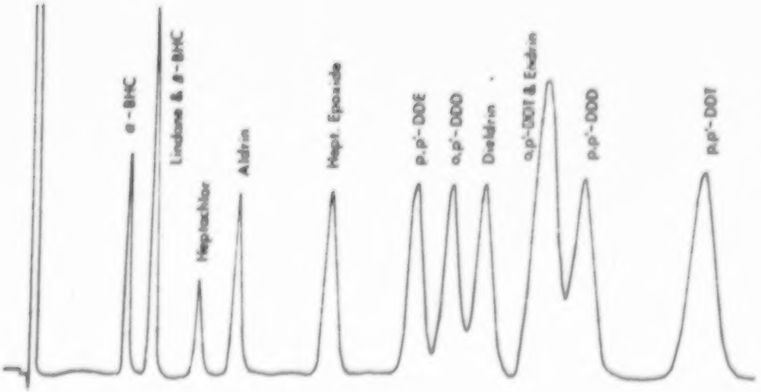


Figure 2

5% OV-210

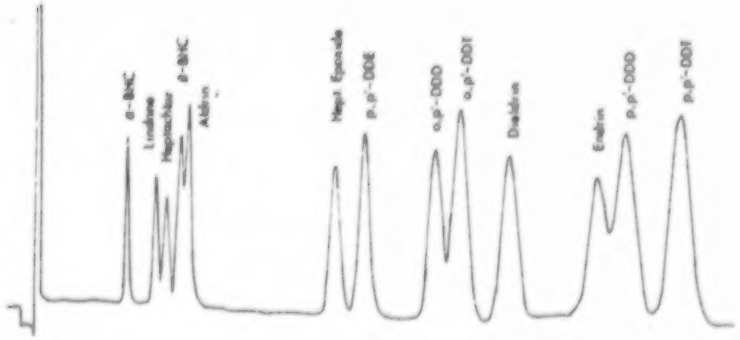


Figure 3

Figure 3a

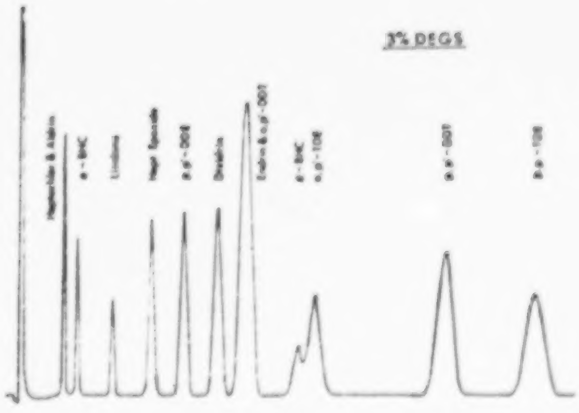


Figure 3b

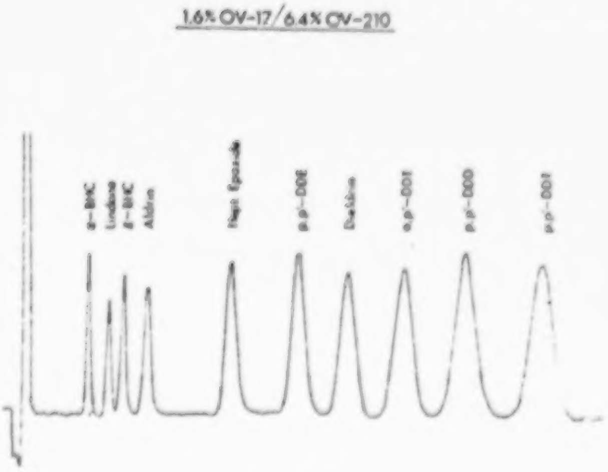


Figure 3c

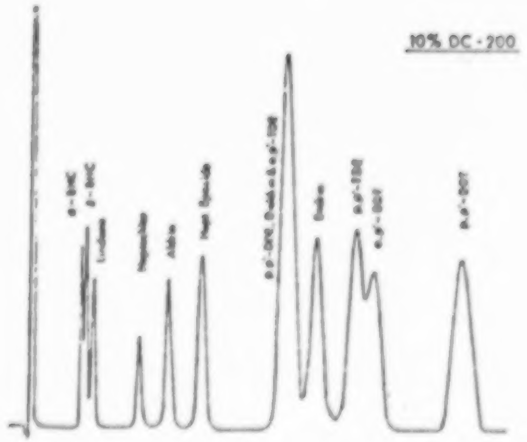


Figure 3d

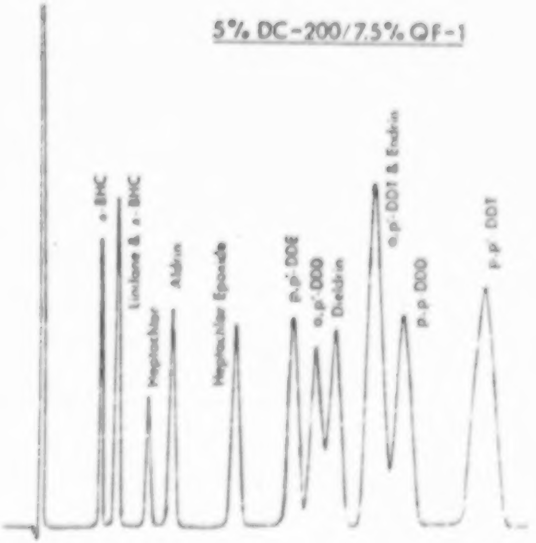


Fig. 4 – Columnto Port Assembly – Exploded View

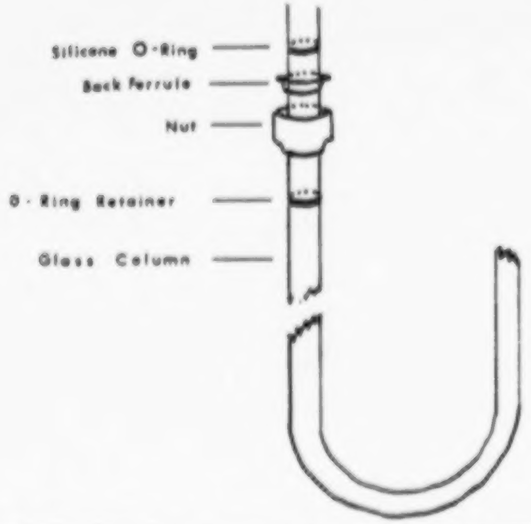

Silicone O-Ring
Back Ferrule
Nut
O-Ring Retainer
Glass Column

Fig. 4 (a) – Bubble Flowmeter

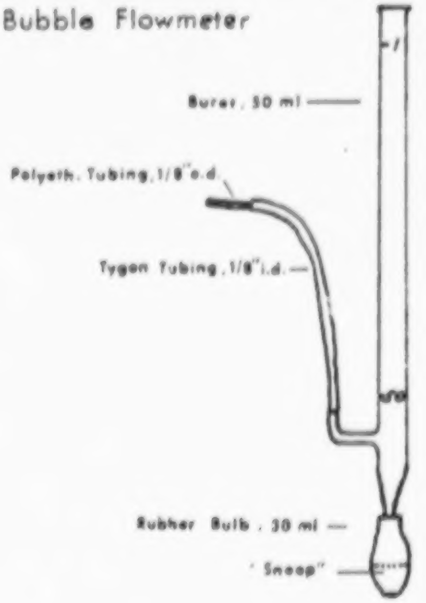
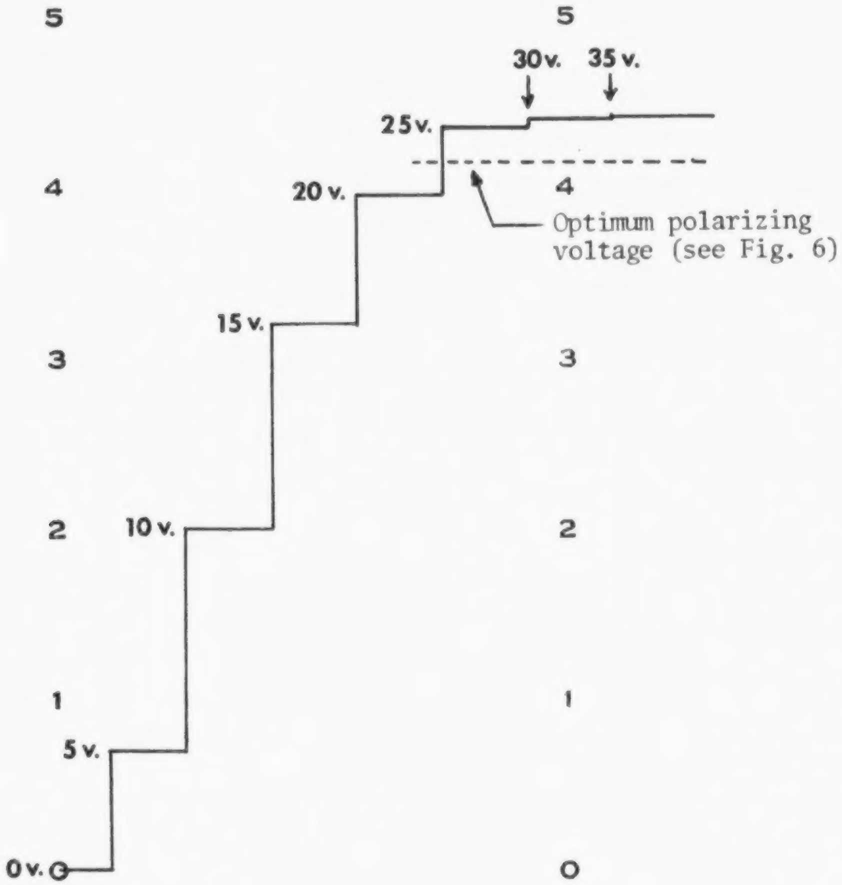

Buret, 50 ml
Polyeth. Tubing, 1/8" o.d.
Tygon Tubing, 1/8" i.d.
Rubber Bulb, 30 ml
"Snoop"

Figure 5. Standing current profile from ^3H detector in constant use 60 days. Instrument Tracor MT-220; electrometer attenuation 10 x 256, detector temp. 200°C., column 3% OV-1, column temp. 180°C., carrier gas nitrogen, flow rate 45 ml/min, purge flow 30 ml/min



Optimum polarizing voltage (see Fig. 6)

Figure 6. Voltage/Response curve for 50 pg of aldrin from ^3H detector in continual use 60 days. Instrument Tracor MT-220; electrometer attenuation 10 x 32; column 3% OV-1, column temp. 180°C., detector temp. 200°C., nitrogen carrier flow 60 ml/min

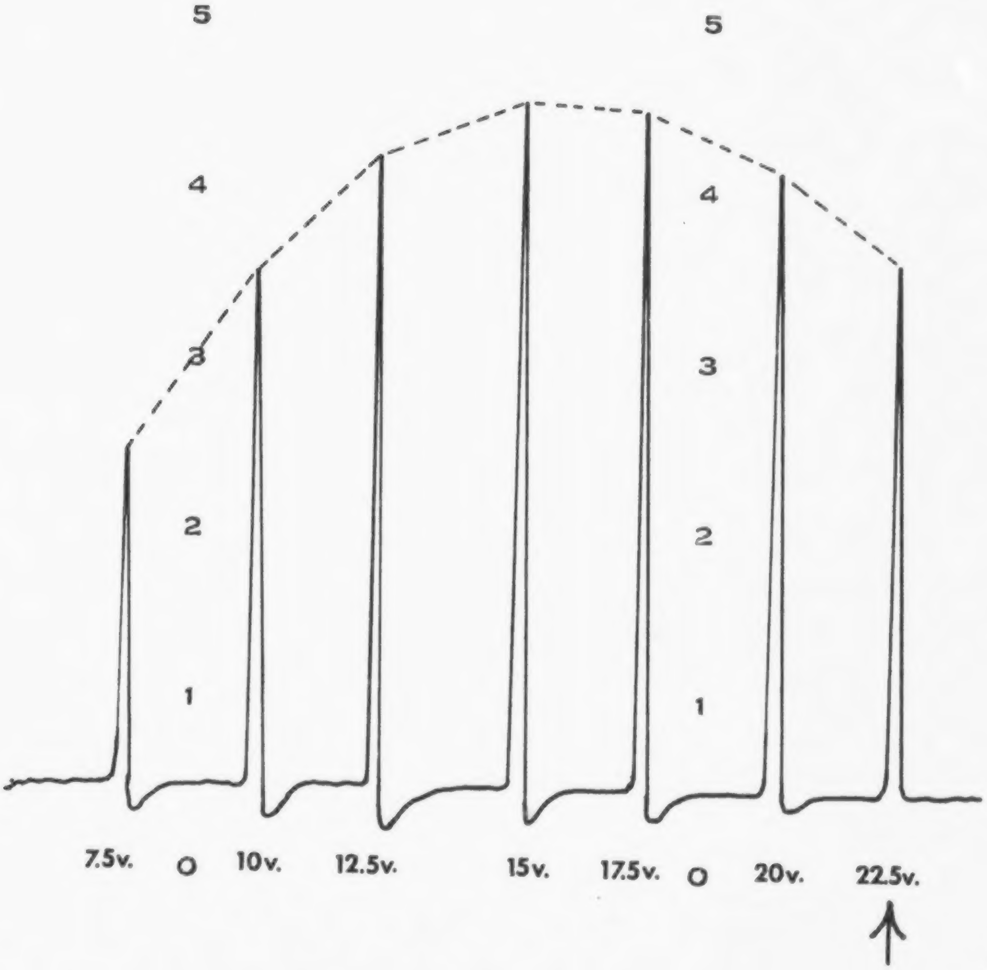


Fig. 7

Peak Height = CD

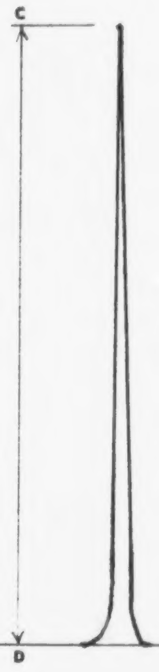
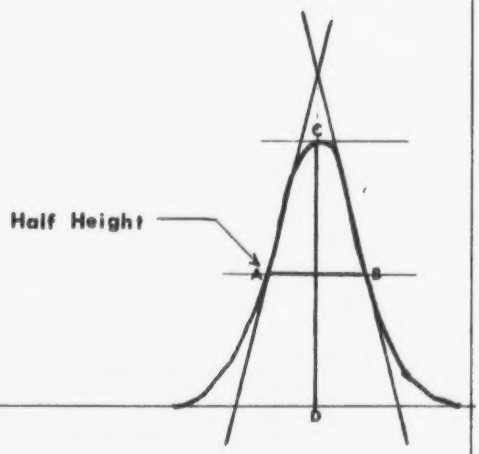


Fig. 8

Peak Area = CD × AB


C
Half Height
A
B
D

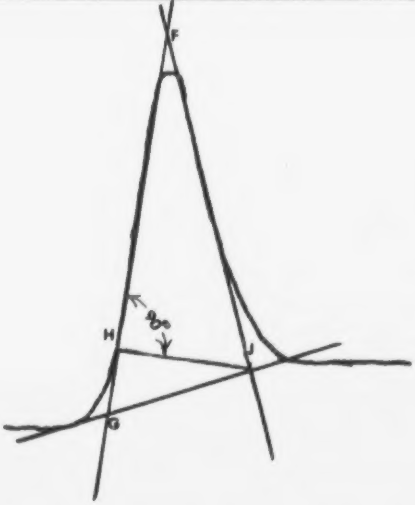


Fig. 9 Triangulation

Peak Area = $\frac{1}{2}$ (FG) (JH)

Fig. 10. Copied from Pesticide Analytical Manual, Vol. 1, U.S. Food & Drug Administration.

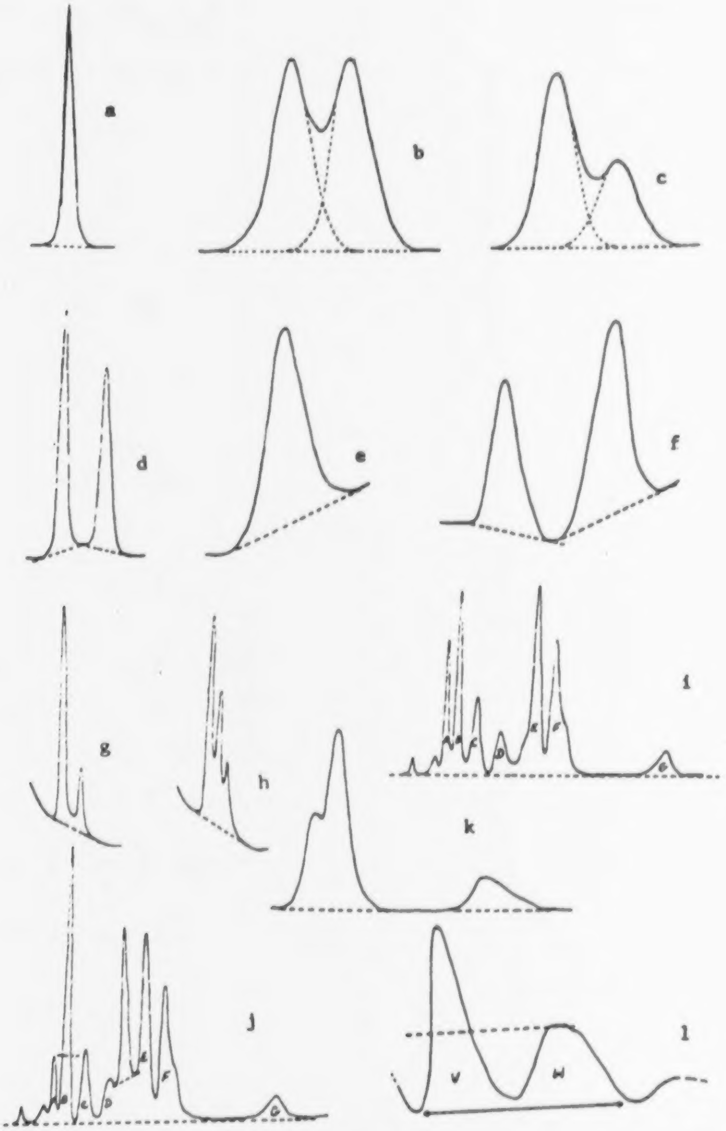
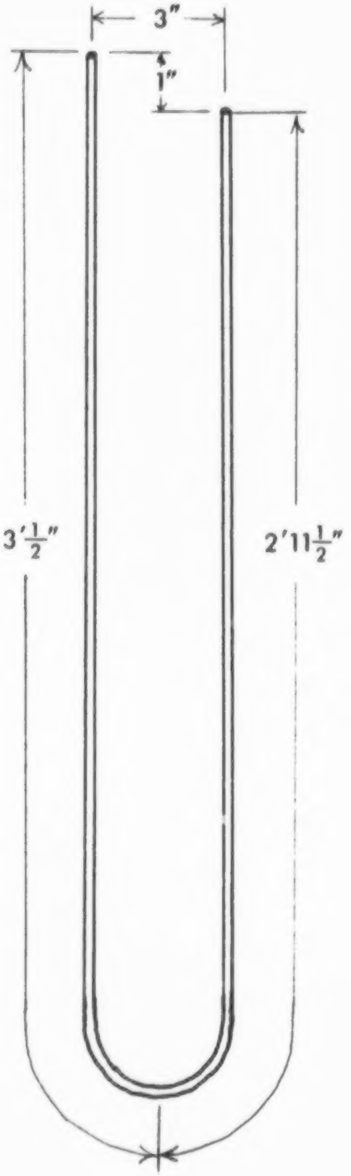


Fig. 2--Baseline construction for some typical gas chromatographic peaks. a, symmetrical separated flat baseline; b and c, overlapping flat baseline; d, separated (pen does not return to baseline between peaks); e, separated sloping baseline; f, separated (pen goes below baseline between peaks); g, α- and γ-BHC sloping baseline; h, α-, β-, and γ-BHC sloping baseline; i, chlordane flat baseline; j, heptachlor and heptachlor epoxide superimposed on chlordane; k, chair-shaped peaks, unsymmetrical peak; l, p,p'-DDT superimposed on toxaphene.

Figure 11. Six-foot gas chromatographic column. Borosilicate glass, 1/4" o.d., 5/32" i.d. - Corning No. 237300 or equivalent. Tubing o.d. to be tested for assurance it will accomodate 1/4" Swagelok nut. (If butt-jointed, the butt to be on one side, not at U-bend.)



SUPPORT-BONDED CARBOWAX 20M COLUMNS

I. INTRODUCTION:

Highly inert column packings have been prepared by chemically bonding Carbowax 20M to different GC supports. The Carbowax is coated on acid washed support, and, after heat conditioning, the nonbonded phase is removed by solvent extraction. A thin layer of liquid phase remains bonded to the support surface. Packings prepared in this way have been used for the GC of chlorinated pesticides without further treatment, and for heat-labile nitrogen-containing and other polar pesticides after being coated with liquid phases such as OV-101 or OV-210. The columns have been used with electron capture (Section 12, A), Hall electrolytic conductivity (Section 4,C), and nitrogen-phosphorus thermionic (Section 4,D) GC detectors for the separation and analysis of pesticides.

The preparation of columns described in this section differs from vapor phase deposition of Carbowax 20M (Section 4,B,(2), IV,2) used to make columns more suitable for the GC of organophosphorus pesticides. This earlier form of support bonding produces only temporary and less complete deactivation and had to be repeated periodically.

REFERENCES:

1. Synthesis and Chromatographic Applications of Bonded, Monomolecular Polymer Films on Silicic Supports, Aue, W. A., Hastings, C. R., and Kapila, S., Anal. Chem., 45, 725 (1973) and J. Chromatogr., 77, 299 (1973).

2. Laboratory Preparation and Applications of Modified Carbowax 20M Bonded Supports to the Gas Chromatography of Pesticides, Winterlin, W. L., and Moseman, R. F., J. Chromatogr., 153, 409 (1978).

3. Rapid Procedure for Preparation of Support Bonded Carbowax 20M Gas Chromatographic Column Packing, Moseman, R. F., J. Chromatogr., 166, 397 (1978).

II. PREPARATION OF SUPPORTS:

Commercial support-bonded Carbowax 20M column packings are costly and have proved to be variable among batches and suppliers. The following laboratory procedure for preparing the packing is much more rapid than earlier methods and produces a highly deactivated, low-bleed material. It is possible for one person to prepare support-bonded and coated column packings for use in less than four days.

1. Wash the commercial support (e.g., Chromosorb W or G) by slurrying with hot 6 N HCl in a 350 ml coarse-frit Buchner funnel. Draw off the acid with vacuum produced by a water aspirator. Repeat the washing until all traces of yellow color are removed; no more than 3 or 4 washings are usually required.

   NOTE: Acid treatment of supports, whether acid washed by the manufacturer or not, greatly improves their chromatographic performance, particularly for many pesticides that are difficult to chromatograph.

2. Wash the support in the funnel with several portions of distilled water to remove excess acid.

3. Oven dry the support at 100°C overnight and then coat with 3-5% Carbowax 20M, using rotary vacuum (EPA Pesticide Analytical QC Manual, Section 4G) or vacuum filtration (Technical Bulletin No. 2A, 1967, Applied Science Laboratories, Inc.; see Section 4,A,(4).

4. Carry out the heat treatment (support bonding) process in a 100 ml volumetric pipet as follows:

   a. Pack the portion of the pipet below the bulb with uncoated support held in place with glass wool plugs. Fill the remainder of the pipet with coated support.

      NOTE: The lower bed of support prevents back diffusion of oxygen into the coated support.

   b. Connect the pipet to the inlet of a Tracor MT-222 or equivalent conventional gas chromatograph, using Swagelok fittings drilled to the proper size and a ferrule fabricated with PTFE to obtain a gas-tight seal between the pipet and fitting (Figure 1).

   c. Sweep nitrogen through the column packing at a flow rate of 60 ml/minute for at least 2 hours at room temperature.

d. Program the temperature of the GC oven to 270°C at 1°C/minute and hold for 16 hours.

e. Cool the oven to room temperature while maintaining the nitrogen flow.

f. Remove the pipet and empty the contents into a 350 ml coarse-frit Buchner funnel.

5. To remove nonbonded Carbowax 20M, slurry the support with the solvent used for coating in Step 3 and draw off the solvent with vacuum into a filter flask.

6. Repeat this process four or five times until two successive washes yield no yellow color.

7. Transfer the packing material to a sheet of aluminum foil and air dry in a fume hood.

8. If the support is to be used without coating, pack into a GC column (Section 4,A,(2),III) and condition for at least 16 hours at 230°C (Section 4,A,(2),IV).

NOTES: 1. Be sure to purge oxygen from the column during the conditioning process before increasing the oven temperature.

2. An occasional reconditioning of columns at 230°-240°C for overnight periods can be beneficial in restoring performance of columns used for analysis of lipid extracts.

3. A new Carbowax 20M column can exhibit a sharp increase in response after injection of a number of lipid-containing extracts. It is probably that the improved column performance is due to coverage of residual active sites on the support.

9. If the support is to be coated with a liquid phase, use any standard coating method, e.g., vacuum filtration (Step 3 above). If fines appear to be a problem, pass the packing through the proper mesh-size screen.

10. If properly prepared and used, columns can be stable for at least several months. Exclusion of oxygen during operation at elevated temperatures is an important factor.

III. APPLICATIONS, CHROMATOGRAMS, AND DATA:

Coating the support-bonded material with OV-101 provides a column packing that allows chromatography of many polar or heat labile compounds such as intact carbamate pesticides, chlorinated anilines, and metabolites of triazine herbicides, usually without derivatization. Figure 2 shows the gas chromatogram for a nine-component mixture of pesticides of different classes on a 3% OV-101 column coated on Chromosorb W support-bonded Carbowax 20M (A) and unbonded Gas-Chrom Q support (B). All nine compounds chromatographed well on the support-bonded packing, while the carbamate pesticides propoxur and carbaryl did not chromatograph on the conventional column. The elution order of eicosane (C_{20}), atrazine, and pentachloronitrobenzene (PCNB) can be seen to vary on the two columns, presumably due to the presence of the small amount of Carbowax. The carbamate pesticides carbofuran, aminocarb, and mexacarbate also chromatographed well on the support-bonded Carbowax 20M packing.

Figure 3 illustrates the separation of two N-dealkyl metabolites of triazine herbicides that could not be chromatographed on conventional column packings. Figure 4 demonstrates the GC of several chlorinated anilines that ordinarily require derivatization prior to chromatography on a methyl silicone liquid phase. The chromatograms shown in Figures 1-4 were all obtained using a packed 1.8 m x 4 mm i.d. glass column and a flameless nitrogen-phosphorus detector.

Twenty-one pesticides varying greatly in polarity and chromatographic behavior on conventional silicone-coated columns were evaluated on modified Carbowax 20M supports with and without OV-210 coating. Many of the chosen compounds are thermally unstable, yield unfavorable separations, and/or give peaks characterized by tailing or broadening. Supports were packed in 1.8 m x 2 mm i.d. U-shaped glass columns, and compounds were detected with a ^3H or ^{63}Ni electron capture detector.

Tables 1 and 2 compare relative retention values and chromatographic efficiency as indicated by peak shape for the 21 pesticides on each of five Carbowax 20M modified supports both with and without OV-210 coating. It was found that Gas-Chrom P and Q bonded with Carbowax 20M gave, in general, the most desirable chromatographic behavior. In addition, coating the modified supports with OV-210 generally altered the relative retention values and improved the chromatographic behavior and separations of the pesticides. Figure 5 depicts a typical improvement in peak shape due to coating a modified support with OV-210.

As expected, considerable improvement in chromatographic behavior was also obtained for Carbowax 20M deactivated supports coated with OV-210, compared to nontreated supports coated with OV-210 (Figure 6).

Carbowax 20M modified supports were also found to offer significant advantages over columns treated with traditional silylating reagents (Section 4,A,(2),IV,2).

All of the data and figures in this section emphasize the importance of a highly deactivated support when attempting the gas chromatography of many polar and labile pesticides of current importance to the analyst.

TABLE 1. PEAK SHAPE AND RELATIVE RETENTION ON CARBOWAX 20M MODIFIED SUPPORTS COATED WITH OV-210

Peak shape is defined by numbers: 1 = sharp peak with little or no tailing; 3 = broad but symmetrical with little or no tailing; 4 = moderate tailing; 5 = severe tailing; N.P. = no peak. RRT = relative retention time (Parathion = 1.00). All column temperatures were held constant at 200.

Pesticide	10% OV-210 on Chromosorb P		10% OV-210 on Chromosorb G		5% OV-210 on Chromosorb W		5% OV-210 on Gas-Chrom P		5% OV-210 on Gas-Chrom Q	
	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT
Phosphamidon	3	0.10	1	0.10,0.12	1	0.08,0.10	1	0.12,0.14	4	0.12,0.15
Mevinphos	3	0.15	1	0.15	1	0.17	1	0.17	1	0.17
Methamidophos	-	-	3	0.15	5	0.17	3	0.15	4	0.15,0.26
Diazinon	3	0.20	1	0.23	1	0.20	1	0.21	1	0.15
Lindane	3	0.23	1	0.24	1	0.23	1	0.25	1	0.26
Disulfoton	3	0.24	1	0.25	1	0.25	1	0.26	1	0.27
Atrazine	3	0.29	1	0.30	1	0.26	1	0.28	1	0.30
Simazine	3	0.33	1	0.34	1	0.28	1	0.29	1	0.32
Benefin	-	-	1	0.25	1	0.28	1	0.29	1	0.28
Trifluralin	-	-	1	0.25	1	0.29	1	0.29	1	0.28
Aldrin	3	0.27	1	0.28	1	0.28	1	0.29	1	0.29
Dioxathion	3	0.28	3	0.30	1	0.29	1	0.31	1	0.32
Chlorpyrifos	3	0.43	1	0.45	1	0.44	1	0.46	1	0.46
Monocrotophos	-	-	3	0.25,0.84	4	0.76	3	0.71	4	0.26,0.74
Methyl Parathion	3	0.79	1	0.80	1	0.78	1	0.80	1	0.80
Parathion	3	1.00	1	1.00	1	1.00	1	1.00	1	1.00
Chlorpyrifos oxygen analogue	-	-	3	0.86	-		1	0.23,1.00	-	
p,p'-DDT	3	1.18	3	1.26	1	1.06	1	1.15	1	0.62,1.17
Paraoxon	N.P.	N.P.	1	1.30	1	1.35	1	1.34	1	1.33
TEPP	-	-	3	1.47	1	1.40	1	1.41	1	1.39
Azinphos-methyl	-	-	1	7.36	1	5.74	1	6.06	3	7.10

'Very poor response with many small peaks and a large hump.

TABLE 2. PEAK SHAPE AND RELATIVE RETENTION ON UNCOATED CARBOWAX 20M MODIFIED SUPPORTS

Peak shape is defined by numbers: 1 = sharp peak with little or no tailing; 2 = sharp but tailing; 3 = broad but symmetrical with little or no tailing; 4 = moderate tailing; 5 = severe tailing; 6 = peak poorly distinguished; N.P. = no peak. RRT = relative retention time (Parathion = 1.00). All column temperatures were held constant at 175° with the exception of Chromosorb P which had a column temperature of 220°.

Pesticide	Chromosorb P		Chromosorb G		Chromosorb W		Gas-Chrom P		Gas-Chrom Q	
	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT	Peak shape	RRT
Phosphamidon	4	0.13	-	0.05,0.07	1	0.08	1	0.52,0.78	1	0.11,0.85
Mevinphos	1	0.21	1	0.12	1	0.16	1	0.14	1	0.14
Methamidophos	4	0.17	4	0.32	4	0.62	3	0.28	4	0.18
Diazinon	3	0.24	3	0.31	1	0.37	1	0.29	1	0.35
Lindane	1	0.27	3	0.26	1	0.24	1	0.31	1	0.31
Disulfoton	1	0.28	3	0.28	1	0.31	1	0.29	1	0.30
Atrazine	4	0.32	3	0.61	4	0.62	1	0.65	1	0.65
Simazine	4	0.33	3	0.72	4	0.79	1	0.82	1	0.86
Benefin	1	0.29	1	0.15	1	0.18	1	0.17	1	0.18
Trifluralin	1	0.29	1	0.14	1	0.18	1	0.17	1	0.18
Aldrin	1	0.32	3	0.25	1	0.26	1	0.26	1	0.28
Dioxathion	1	0.32	3	0.35	4	0.36	3	0.37	1	0.38
Chlorpyrifos	1	0.46	3	0.59	1	0.60	1	0.55	1	0.56
Monocrotophos	4	0.78	4	0.16,1.48	5	2.22	2	1.49	2	1.46
Methyl Parathion	1	0.82	3	0.84	1	0.72	1	0.90	1	0.90
Parathion	1	1.00	3	1.00	1	1.00	1	1.00	1	1.00
Chlorpyrifos oxygen analogue	6	-	6	-	6	-	1	0.78	1	0.72,1.84
p,p'-DDT	3	1.11	3	2.32	3	0.82,1.99	1	2.39		2.34
Paraoxon	N.P.	-	3	1.06	4	1.35	1	1.04	1	1.06
TEPP	1	2.36	3	2.90	1	3.17	1	2.51	1	2.58
Azinphos-methyl	N.P.	-	3	12.06	3	10.17	1	13.00	1	11.75

[*] Peaks on a solvent front; could not distinguish peak shape.

PREPARATION OF SUPPORT-BONDED CARBOWAX 20M GC PACKING

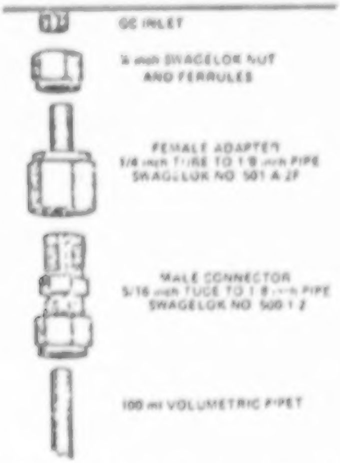

GC INLET
¼ inch SWAGELOK NUT
AND FERRULES
FEMALE ADAPTER
1/4 inch TUBE TO 1/8 inch PIPE
SWAGELOK NO 501 A 2F
MALE CONNECTOR
5/16 inch TUBE TO 1/8 inch PIPE
SWAGELOK NO 500 1 2
100 ml VOLUMETRIC PIPET

FIGURE 1. Swagelok adapter for heat treating in a GC oven.

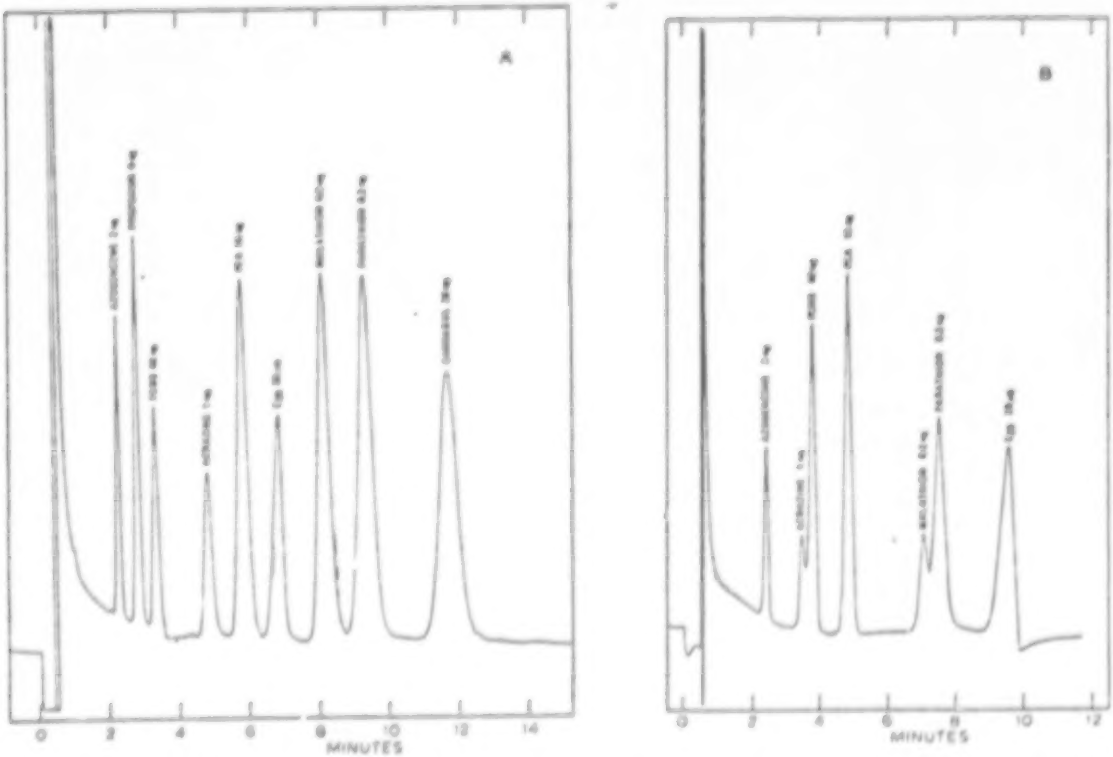


FIGURE 2. (A) Gas chromatogram of nine-component mixture on 3% OV-101 coated on 80-100 mesh Chromosorb W support-bonded Carbowax 20M. (B) Same mixture on 3% OV-1 coated on 80-100 mesh Gas-Chrom Q. Column temperature, 185°; helium flow-rate, 57 ml/min. Perkin-Elmer N-P detector, nitrogen mode.

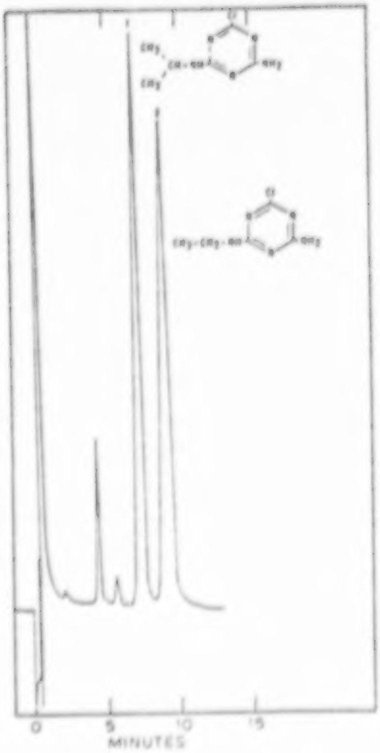


Figure 3.

Gas chromatogram of N-dealkyl metabolites of triazine herbicides with same conditions as in Fig. 2A.

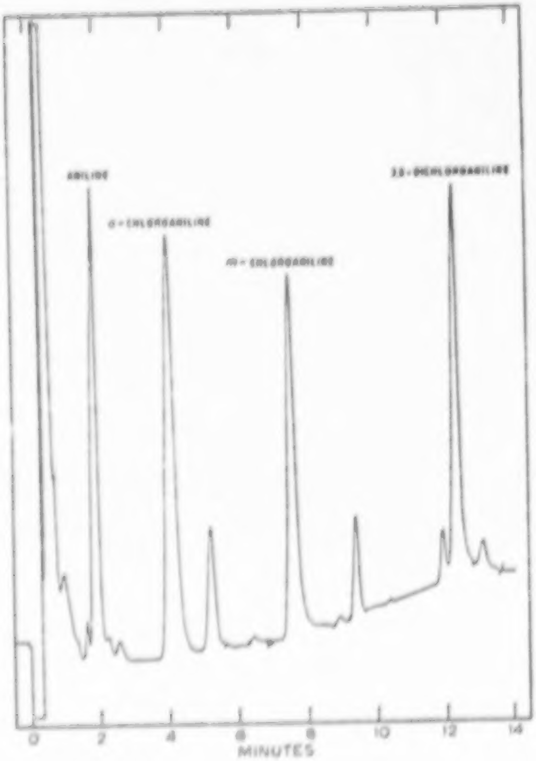


Figure 4.

Gas chromatogram of chlorinated anilines on 2% OV-101 coated on 80-100 mesh Chromosorb W support-bonded Carbowax 20M. Column temperature programmed from 100° to 160°C at 8°C/minute; 4 minute initial hold.

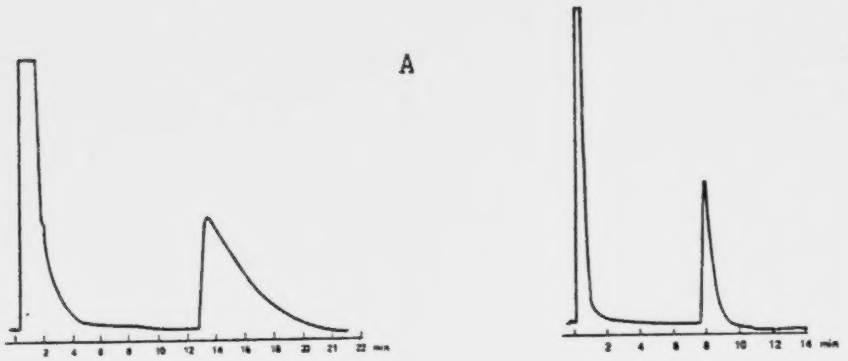


Fig. 5. Chromatograms of monocrotophos on Carbowax 20M modified Chromosorb W support before (A) and after (B) coating with 5% OV-210. Column temperature 175°C in (A) and 200°C in (B).

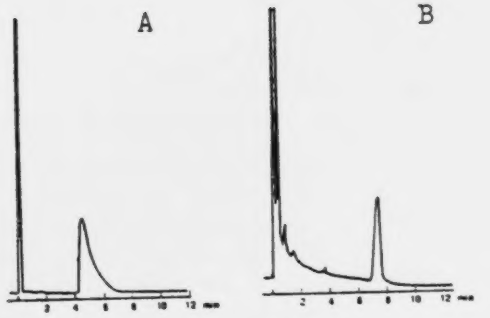


Fig. 6. Chromatograms of disulfoton on (A) nontreated Chromosorb P coated with 10% OV-210 and (B) Carbowax 20M modified Chromosorb coated with 10% OV-210. The column temperature was 200°C in both cases.

TECHNICAL BULLETIN No. 2A

Applied Science
Laboratories, Inc.
P.O. Box 440 / State College, Pennsylvania 16801

Preparation of Coated Packings

Use of the HI-EFF FLUIDIZER

This bulletin describes the procedures used in making coated packings by means of the HI-EFF Fluidizer*. Although the procedures described in this bulletin may at first glance appear complicated, with a little study and practice packings of the highest quality can be easily and quickly made (1).

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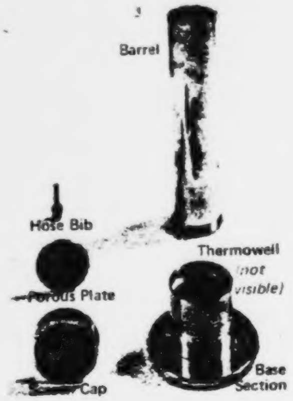
I. DESCRIPTION OF THE FLUIDIZER

If a gas is passed upward through a porous plate covered with a finely divided solid, the particles are suspended and intimately mixed in the gas stream. The suspended particles exhibit fluid characteristics and the process is called fluidization. This process provides an ideal method for drying packings intended for gas chromatography. Fluidization drying prevents clumping of the packing and insures uniform coating of the support with stationary phase. The basic parts of the HI-EFF Fluidizer are shown and labeled in the exploded view. Base section, cap, and barrel are made of chrome-plated brass. The screen cap prevents the packing from blowing out of the barrel if the gas rate is initially too high. The barrel screws into the base section and clamps the porous plate in position. The base is designed with a spiral insert (silver soldered in place) which provides good heat transfer characteristics for preheating the gas when the Fluidizer is set on a hot plate. The base also contains a 6 mm thermowell to permit measurement of gas temperature. An interchangeable hose bib and a compression fitting are included with the unit, permitting the use of either metal or rubber tubing to connect the Fluidizer to a gas cylinder.



The unit is already assembled when received but can be stripped down to its basic parts simply by unscrewing the barrel from the base, exposing the porous bronze plate. The plate rests on a flange and drops out when the base is inverted.

II. CLEANING

The Fluidizer can be cleaned simply by blowing out residual packing with compressed gas, but for thorough cleaning the unit should be disassembled and the barrel, cap, and porous plate soaked in a suitable solvent. Clean the base section with the solvent used for the phase if a solution of phase has inadvertently been poured into the unit without gas flow. WARNING: If this happens when the base is heated, the base can become plugged and the porous plate ruined.

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*U.S. Patent 3, 513, 562

(1) Kruppa, R.F., R.S. Henly, and D.L. Smead, Improved Gas Chromatography Packings with Fluidized Drying, Anal. Chem. 39, 351 (1967)

III. FLUIDIZATION GASES

Nitrogen is recommended for the fluidization gas because it is inert and inexpensive, although compressed air can be used for phases which are not sensitive to oxidation. A HYDRO-PURGE® or similar molecular sieve trap can be inserted in the gas line between the cylinder and Fluidizer, if desired. A cylinder reducer valve for the 0-50 psig range is recommended, although a 0-250 psig valve may be used if **great care** is taken when turning on the fluidizing gas.

IV. PRECAUTIONS

This unit may be used to prepare almost every type of packing used for gas chromatography columns **except** those containing highly corrosive materials such as silver nitrate, strong acids (H_3PO_4, H_2SO_4, etc.), strong bases (NaOH, KOH, etc.) or corrosive organics.

There is no need to operate this unit above 150°C (its temperature limit). Discoloration of the chrome plating on the base can be expected from prolonged usage or excessive high temperature exposure but this will in no way interfere with the operation of the unit. The barrel should be replaced if it becomes badly discolored, although this is unlikely to happen if the temperature limit is observed.

V. GENERAL OPERATION

A small amount (50 g) of GAS-CHROM S is supplied with the HI-EFF Fluidizer. To become familiar with the operation of the unit it is suggested that the user practice with this support by carrying the procedure through the filtration stage (Section VII) with solvent alone (a stationary phase is not necessary in this case). A maximum of 50 g of packing can be made with one filling of the Fluidizer. Heat is supplied to the packing by preheating the fluidizing gas. This is easily accomplished by setting the Fluidizer on a hot plate (any type available in the laboratory is satisfactory). Heat from the hot plate is transferred to the gas as it flows through a long spiral path in the base of the Fluidizer and emerges below the porous plate. Gas temperature can be measured by inserting a laboratory thermometer in the well in the base. The heat supply should be controlled by operating the hot plate through a laboratory Variac (See Section VI). In this way the gas temperature below the porous plate can be maintained within a 5°C range.

In general, good packings can be produced with gas temperatures in the range of 40 to 100°C. A good rule of thumb is to operate with the gas temperature just below the boiling point of the solvent being removed. With solid stationary phases best results appear to be obtained with gas temperatures below the melting point of the phase. **The Fluidizer will operate correctly with gas pressures of 1 to 2 psig.**

VI. TEMPERATURE CALIBRATION

Although the gas temperature can be measured while drying is in progress, we recommend that a simple temperature versus Variac voltage calibration plot be made initially with the user's hot plate. This allows the voltage to be preset when a packing is to be dried.

To carry out the calibration, place about 25 grams of dry support (as supplied) in the Fluidizer, place the Fluidizer on the hot plate, and apply a low heat (about 20 volts Variac setting). After 10 minutes gradually start the gas flow and set a rate which will result in gentle fluidization of the support (See Notes 1 and 2 in section VII). Take successive temperature readings at 5 minute intervals until two consecutive readings agree within 5 degrees. Record this temperature and voltage. Raise the voltage in several increments until a gas temperature of 100°C is reached, record the temperature for each voltage setting, and make a plot of gas temperature versus Variac voltage.

Use of this plot without measuring temperatures while drying should be satisfactory. However, if desired, the gas temperature can be measured during operation and a finer control of temperature obtained by small adjustments of voltage. Remember: Do not exceed 150°C.

VII. FILTRATION-FLUIDIZATION PROCEDURE

Do not start this procedure until you have read the instructions all the way through. The notes are particularly important.

The following symbols will be used in this description:
a = number of grams of support to be coated
b = desired percent (w/v) of stationary phase, divided by 100

Step 1 Weigh *a* grams of support into a beaker or similar container. Weigh 5*ab* grams of stationary phase in another beaker (Note 3). Measure 5*a* ml of solvent in a graduated cylinder and add to the stationary phase (Note 4). Completely dissolve the liquid phase in the solvent by stirring with a glass rod or with a magnetic stirrer. Minimize evaporation of the solvent so that stationary phase concentration will not be changed significantly.

Step 2. Place the HI-EFF Fluidizer on the hot plate, turn on the Variac and allow the unit to warm up without gas flow while performing Step 3. Step 3 and warm-up of the Fluidizer both require about 10 minutes and should proceed simultaneously.

Step 3. Slowly add the support to the solution of stationary phase while stirring constantly but gently with a glass rod or other convenient device. Let the support settle for 5 minutes (Note 5), then slurry again by gentle stirring (Note 6). Immediately pour the cream-like mixture into a Buchner funnel mounted on a vacuum filter flask. Apply vacuum to the flask with an aspirator and filter off the excess solution. Continue filtration until solution ceases to drip from the funnel.

Step 4. Transfer the damp packing to the Fluidizer through a wide mouth funnel by inverting the Buchner funnel over the Fluidizer and tapping gently with the heel of of the hand. Slowly turn on the fluidizing gas and closely observe the packing. It will rise upward as a solid plug and the proper gas setting can be made by allowing only a 1/4 to 1/2 inch rise of the upper surface of the plug of packing in the barrel (Note 1).

Step 5. Put the cap in place and leave the unit alone for 2 minutes. After 2 minutes the packing should begin to fluidize. At this point it may occasionally be necessary to give the Fluidizer a vigorous shake in order to start fluidization. If the fluidization is too violent, decrease the gas flow rate (Note 2). The packing is dry when no odor of solvent can be detected in the effluent gas stream at the top of the Fluidizer. The total drying time will be 5 to 15 minutes, depending on the amount of packing and volatility of the solvent used in making the packing.

Step 6. Pour the finished packing from the Fluidizer by grasping the barrel near its top (Note 7). Set the Fluidizer aside to cool and clean it with compressed gas or solvent as described under Section II.

Notes and Sample Calculations for Filtration-Fluidization Procedure

Note 1. When gas flow is started, the gas pressure should be raised slowly and carefully. If too much pressure is suddenly applied, the slug of wet packing will blow out the top of the Fluidizer.

Note 2. If the gas rate is too high after fluidization of the packing starts the packing will impinge on and cling to the underside of the screen cap. This is readily observed if it occurs. In this case, decrease the gas rate and remove the cap temporarily to observe the packing. When fluidization is correct, the packing will look like a gently boiling liquid. The gas rate can be adjusted accordingly, but pressures in the range of 1 to 5 psig will give the desired results.

Note 3. The Fluidizer is limited to 50 gram charges. In most cases a b% (w/v) solution of stationary phase will result closely in a b% (w/w) coating on the packing (as in Sample 1 under Note 8). The most notable exceptions are the silicone gums such as OV-1, JXR, SE-30, W-98, etc. In general, a b% (w/v) solution of these silicones will make a 2b% (w/w) coating. Thus a 1.5% (w/v) solution of OV-1 will produce a 3% (w/w) coat (see Sample 2 under Note 8).

Polyester and organosilicone phases tend to coat slightly high at high phase loadings. Thus a 3% (w/v) solution will produce a 3% (w/w) coat, a 10% (w/v) solution will produce an 11% (w/w) packing, and a 16% (w/v) solution results in a packing with about 19% (w/w) coat. See Sample 3 under Note 8.

The best method for determining phase concentration coated on the support is to perform a Soxhlet extraction on a portion of the packing. If this is done over a range of concentrations, a calibration curve can be produced. See Parcher, J.F., and P. Urone, J. Gas Chromatog. 2, 184 (1964).

A simpler alternative, although not exact, is based on the solution retained by the packing during filtration. There is a good indication that the amount of phase coated on the packing is directly proportional to the volume of solution remaining on the support after the filtration step.

Let F = the filtrate volume in ml,
then 5a − F = S ml solution retained on support
and % coat = 100Sb/(a+Sb)

In this laboratory the above method has been found to be quite accurate for large (i.e. 500 g) batches of packings. The inaccuracies encountered with small scale batches depend on the evaporative losses of the solvent. Sample calculations are shown in Note 8.

Note 4. For support mesh sizes of 80/100 or higher, a ratio of solvent to support (v/w) of 5 is used. For mesh sizes below 80/100, such as 60/80, the ratio should be 6. Therefore 6a ml of solvent and 6ab grams of stationary phase should be used for mesh sizes of 60/80 or below. See Sample 3 under Note 8 for the latter case.

Note 5. At the end of 4 minutes it is advisable to remove all particles which float on the surface of the solution. These particles are not wet by the solution and therefore are not coated. They can be skimmed off the surface with an eyedropper inserted into a piece of rubber vacuum tubing connected to the filter flask to be used for filtration of the packing. By applying a vacuum to the filter flask the particles are drawn into the flask. Minimize the amount of solution removed and do not empty the filter flask if you plan to estimate the percent coating by using the filtrate measurement technique described in Note 3.

Note 6. When stirring, do not rub or abrade the packed-down support. Stir the solution vigorously, starting at the top of the support and working down slowly until all the support is slurried.

Note 7. The base and lower part of the barrel become warm during normal operation. However, the top of the barrel should remain cool enough to hold unless the temperature limit is exceeded.

Note 8. Sample calculations for this procedure

Sample 1. Packing desired = 10% DC 200 on 80/100 GAS-CHROM Q

a = 25 g GAS-CHROM Q
5a = 125 ml $CHCl_3$
b = 10/100 = 0.10 g/ml
5ab = 125 (0.10) = 12.5 g DC 200 to be used

After filtration
F = 95 ml
S = 5a − F = 125 − 95 = 30 ml

$$\frac{100\,Sb}{a + Sb} = 100 \cdot \frac{(30)(0.10)}{25 + (30)(0.10)} = 10.7\%\ \text{coating}$$

Sample 2. Packing desired = 3% OV-1 on 100/120 GAS-CHROM Q

a = 50 g GAS-CHROM Q
5a = 250 ml $CHCl_3$
b = 1.5/100 = 0.015 g/ml
5ab = 250(0.015) = 3.75 g of OV-1 to be used

After filtration
F = 150 ml
S = 5a − F = 250 − 150 = 100 ml

$$\frac{100Sb}{a + Sb} = 100 \cdot \frac{(100)(0.015)}{50 + (100)(0.015)} = 2.9\%\ \text{coating}$$

Sample 3. Packing = 15% EGSS-X on 60/80 GAS-CHROM P

a = 25 g GAS-CHROM P
6a = 150 ml $CHCl_3$
b = 13/100 = 0.13
6ab = 150(0.13) = 19.5 g EGSS-X to be used

(calculation continued on page 4)

After filtration
$F = 115$ ml
$S = 6a - F = 150 - 115 = 35$ ml

$$\frac{100Sb}{a + Sb} = 100 \cdot \frac{(35)(0.13)}{25 + (35)(0.13)} = 15.4\% \text{ coating}$$

VIII. SLURRY-FLUIDIZATION PROCEDURE

The quality of packings prepared by this technique can equal that of packings prepared by the Filtration-Fluidization technique described in the previous section. However, great attention to detail during the preliminary drying step is essential to achieve the best possible packing.

Step 1. Select the desired number of grams of packing to be made, e.g. 50.0 g (see example at end of section). Multiply this number by the percent phase coat desired, expressed as a decimal (i.e. 0.03 for 3%). Carefully weigh out in a beaker the amount of phase calculated. Subtract this weight from the grams of packing desired to get the weight of support. Weigh out the support in a separate beaker. Dissolve the phase in a volume of solvent equal (in ml) to 3.5 times the weight of packing desired, then add the support slowly to the solution (with constant stirring) to form a slurry. It may be necessary to add additional solvent to complete the slurry. The final slurry should have the consistency of thick cream. Transfer the slurry to a flat dish and dry slowly under an infrared lamp or on a *warm* hot plate.

Step 2. When the transfer of the slurry to the dish is complete, excess solution can readily be seen. Solvent from the excess solution must be evaporated during this step, but the packing must still be wet when transferred to the Fluidizer. While it is drying the packing must be stirred constantly and gently to maintain uniformity. The packing is ready for the Fluidizer when it looks like wet sand or cottage cheese. No solution should be evident, but the packing should still be quite wet and sticky. The wet packing is now transferred to the preheated Fluidizer. The transfer should be made with some gas flowing through the Fluidizer. If the packing is too wet when it is placed in the Fluidizer the porous disc may be plugged by the excess solution. An inexperienced operator will tend to transfer the packing either too soon or too late during the preliminary drying step. Step 2 is the critical step in this procedure.

Step 3. Final drying by fluidization is now accomplished as described in Steps 4, 5, and 6 of the Filtration-Fluidization procedure (Section VII). The Slurry-Fluidization procedure is not recommended for Teflon supports. A sample calculation for this procedure is given below.

Packing desired = 50 grams coated with 10% SE-30
Weight of SE-30 = 0.10 × 50.0 = 5.0 g
Weight of support = 50 − 5 = 45.0 g
Dissolve the SE-30 in 50.0 times 3.5 or approximately 175 ml of chloroform. For all slurry coatings the exact amount of phase and support is calculated as above.

IX. TEFLON® COATING PROCEDURE

Excellent uniform coated packings may be prepared from Teflon supports by the Filtration-Fluidization procedure. The method is the same as that described in Section VII but the Fluidizer is not heated. Consequently Step 2 of Section VII is omitted. The damp Teflon packing is transferred from the funnel to the Fluidizer in a manner identical to that described earlier for regular GC packings. Immediately after transferring the damp Teflon packing to the Fluidizer the fluidizing gas is turned on. Solvent evaporation will cool the Fluidizer, Teflon, and phase to below the transition temperature of Teflon (19° C). This cooling will permit fluidization of the material without undue aggregation of the particles (lump formation). After 5 minutes the Teflon packing should be fluidizing. If not, a vigorous shake of the Fluidizer will start the process. When the odor of solvent is no longer evident (usually after 20 to 25 minutes) the packing is finished and can be poured into a plastic bottle for storage. Do not attempt to scrape out the material which adheres to the walls or cap of the Fluidizer, because it is unevenly coated and aggregated. Use caution when cleaning the unit after working with Teflon because of its tendency to stick to the unit at room temperature.

We suggest that you use highly volatile solvents for Teflon coating. Such solvents as methylene chloride, acetone, ether, and chloroform seem to work best.

For more information about working with Teflon as a support see: Kirkland, J.J., *Anal. Chem.* **35**, 2003 (1963) and Applied Science's GAS-CHROM Newsletter 8, No. 3 (July 1967). Reprints of both articles are available from Applied Science.

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HI-EFF® is a registered trademark of Applied Science Laboratories, Inc.

X. OTHER DRYING TECHNIQUES

If the Fluidizer is not to be used as the drying method, the basic procedures outlined in this bulletin can still be followed. The other drying methods are well described in these references.

Horning, E.C., Moscatelli, E.A., Sweely, C.C., *Chem. and Ind.* (London), 751(1959)
Zubyk, W.J., Conner, A.Z., *Anal. Chem.* **36**, 912(1960)
Wotiz, H.H., Chattoraj, S.C., *Anal. Chem.* **36**, 1467(1964)
Purnell, Howard, "Gas Chromatography," Wiley, New York, 1962, p. 240

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC INSTRUMENT

I. INTRODUCTION:

Some of the instructions in this section apply specifically to the Melpar flame photometric unit marketed by Tracor, Inc. Other guidelines are broadly applicable, irrespective of the make of the gas chromatograph.

It should be borne in mind that the selection of a proper combination of operating parameters is critical for operation at maximum sensitivity and minimum noise; therefore, the following guidelines should be carefully considered for the achievement of these objectives.

II. FLOW SYSTEM:

The reader is referred to page 1 of Section 4,A,(1). Flow systems should be tight but the F.P.D. is not as sensitive to leaks as electron capture detection.

In addition to the carrier gas, the F.P.D. requires Hydrogen, Oxygen and possibly, air to operate. Leaks in these systems can be hazardous from the explosion standpoint.

III. DETECTOR:

This subject is covered in detail later in Section 4,B,(3).

IV. ELECTROMETER:

See Section 4,A,(1), III. The electrometer for the F.P.D., must deliver at least 750 VDC, and should be capable of delivering at least 1×10^{-6} amperes bucking current.

V. TEMPERATURE PROGRAMMER:

See Section 4,A,(1), IV.

VI. PYROMETER:

See Section 4,A,(1), V.

VII. MISCELLANEOUS:

See Section 4,A,(1), VI.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC

COLUMNS

I. SPECIFICATIONS:

The specifications given in Section 4,A,(2), page 1 should be reviewed.

II. COLUMN SELECTION:

A. 4% SE-30/6% OV-210 - liquid phases premixed and coated on silanized support, 80/100 mesh.

B. 5% or 10% OV-210 - coated on silanized support, 100/120 mesh. See Section 4,A,(2), II.

III. PACKING THE COLUMN:

See Section 4,A,(2) for details.

IV. COLUMN CONDITIONING:

1. Heat condition a 6-foot column of 4% SE-30/6% OV-210 (QF-1) according to Section 4,A,(2), IV,1.

2. It has been determined that a Carbowax deposition treatment will significantly enhance the F.P.D. response of GLC columns comprised of Chromosorb W, H.P. as the support. The treatment has not appeared to produce any difference in columns of Gas-Chrom Q support. The Carbowax treatment outlined differs slightly from the method reported by Ives and Guiffrida[1] in that the 10% Carbowax is packed directly into the front end of the column in the published procedure. In the following procedure, the 10% Carbowax is contained in a short piece of extension tube, and attached to the front end of the column, thus leaving the front end portion of the GLC column undisturbed.

   a. Place a small wad (ca 1/2 in.) silanized glass wool in one end of a 3 in. length of 1/4 in. o.d. glass tubing. Pack loosely with 2 inches of 10% Carbowax and place another wad of glass wool in the other end of the tube (Figure 1).

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[1] Gas-Liquid Chromatographic Column Preparation for Adsorptive Compounds, Ives and Guiffrida, JAOAC, 53, 5, 1970, 973-977.

b. Place Swagelok nut, ferrule and "O" ring on each end of the packed tube. Pass one end of the tube half way through the male union and attach Swagelok nut to union as tight as possible.

c. Attach other end of tube to the column inlet port in the oven, tightening Swagelok nut as much as possible. Place 1/4 in. nut on inlet end of the 6-ft. GLC column (previously heat conditioned) and insert into the male union until it touches bottom end of 3 in. tube, then slack off very slightly to prevent glass ends from grinding together when nut is tightened (Figure 2).

d. Thoroughly tighten Swagelok nut to attach GLC column to male union and place some object on the floor of the oven to function as a retainer in case GLC column should slip out of the union during conditioning period. A Little Jack works nicely. Bring oven heat up to 230° to 235°C and apply a carrier gas flow of 20 ml/min. Hold for a 17-hour period.

NOTES:

1. The combined parameters of temperature, time and carrier flow are critical in the assurance of uniformity of vapor phase deposition as affecting ultimate retention characteristics.

2. Special materials needed include:

   (a) A 3-in. length of borosilicate glass tubing, 1/4 in. o.d. x 5/32 in. i.d. (or 6 mm x 4 mm).

   (b) Silanized glass wool.

   (c) 10% Carbowax 20M on a silanized support, 10/100 mesh.

   (d) Swagelok male union #400-6, 1/4 in. This must be drilled out to accommodate the 1/4 in. o.d. tubing.

3. DO NOT USE A SILYLATED COLUMN WITH F.P.D. The Silyl-8 will bleed into the F.P.D. and fog the heat shield excessively.

4. See Section 4,A,(7) for a more permanent and deactivating Carbowax treatment.

V. EVALUATION OF COLUMN:

See Section 4,A,(2), V for general evaluation guidelines.

After overnight equilibration, recheck the oven temperature and carrier gas flow rate. For optimum performance, it is advisable at this point to adjust all operating parameters.

A. See the F.P.D. operation manual for a schematic of the recommended gas flow pattern to the detector. Typical approximate gas flows are as follows:

Nitrogen carrier	70-80 ml/minute
Hydrogen flow	50-100 ml/minute
Oxygen content of air flow	0.2-0.4 of the hydrogen flow
Total air flow	1.5 times the hydrogen flow

Example:

Hydrogen flow = 60 ml/minute

60 ml x 0.3 = 18 ml/minute oxygen needed

18 ml ÷ 0.20 (% O_2 in air) = 90 ml/minute air required

NOTES:

1. If the F.P.D. and F.I.D. are in operation simultaneously, the oxygen-to-hydrogen ratio should be closer to 0.4, which will result in decreased sensitivity for the F.P.D. response.
2. Higher gas flows will increase background noise.

B. Suggested approximate operating temperatures are as follows:

Column	200°C
Injection block	225°C
Detector	175-225°C
Transfer line	235°C

NOTES:

1. Do not heat the column until the detector has reached operating temperature.

2. The F.P.D. will operate within the range of 120-250°C, but do not heat above 250°C or damage to the plastic photomultiplier tube housing may result.

C. When flow and temperature parameters have been adjusted to produce optimum signal-to-noise ratio, baseline noise should not exceed 2.5% FSD, and an injection of 2.5 nanograms of parathion should result in a peak of at least 50% FSD.

The following mixture should produce approximately equal peak heights of at least 10% FSD.

Compound	ng
Ethyl Parathion	0.50
Methyl Parathion	0.38
Diazinon	0.17
Ronnel	0.25
Malathion	0.51
Trithion	1.22
Ethion	0.58

NOTES:

1. A drastic reduction in the peak height of malathion can be an indication of a poor column, provided that the rest of the system is known to be operating properly.

2. With some organophosphorus compounds, it will be necessary to make several consecutive injections with fairly high concentrations of the compound to achieve adequate and reproducible response. This is an important consideration if quantitation is to be conducted.

VI. MAINTENANCE AND USE OF COLUMN:

See Section 4,A,(2), VI.

The effects of the vapor phase deposition from Carbowax appear to persist at least three months with a slow decrease in response becoming evident, depending on the particular column and the amount and type of use.

Each operator should monitor the response characteristics in relationship to the column just after treatment.

NOTES:

1. Response will sometimes decrease rapidly for several days after treatment, then stabilize.
2. Retreatment of columns appears to rejuvenate the response but may result in shifts of some of the RRT-P values. See Table 1, Section 4,B,(5). Retreatment is not advised, therefore, because the data contained in Tables 2, 3, and 4 would become unusable.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC DETECTOR

I. OPERATING PARAMETERS:

DC voltage should be supplied to the detector from either an outboard power supply or from a strip on the back of the electrometer. Provided the column and all electronic circuits in the various modules of the instrument are functioning properly, the degree of sensitivity in the flame photometric mode relates to four factors: (1) condition of the photomultiplier (P.M.) tube; (2) voltage applied to P.M. tube; (3) flow rates of hydrogen, oxygen and air; and (4) condition of interior of detector.

II. OPTIMUM RESPONSE VOLTAGE:

In order to determine the optimum response voltage for the P.M. tube, a variable power supply is necessary which allows the voltage to be increased with little increase in electronic noise. Increasing the voltage from the electrometer will increase the electronic noise inordinately.

1. Optimize all temperature and flow parameters as described in Section 4,B,(2).

2. With flows at optimum, set power supply at 750 V DC. Inject enough ethyl parathion to give 35 to 60 FSD.

3. Reset voltage to 850 V. Inject the same amount of ethyl parathion as before.

4. Repeat in 100 V increments until the signal-to-noise ratio reaches maximum and starts to decrease (Figure 4).

NOTES:

1. It should be necessary to attenuate to keep on scale. It is therefore mandatory to check the linearity of the electrometer at different attenuations.

2. Comparison with a P.M. tube of known sensitivity will give indication of condition of P.M. tube.

III. DETECTOR LINEARITY:

The FPD has a broad range of linearity for phosphorus. Excluding any effect from the instrument electronics, the effective range is from 1 to 50 times the minimum acceptable level of ethyl parathion (0.5 ng to 25 ng). The appropriate attenuation will depend on the sensitivity of the particular system used. It is best to operate at the minimum detection level and dilute the sample when necessary, however.

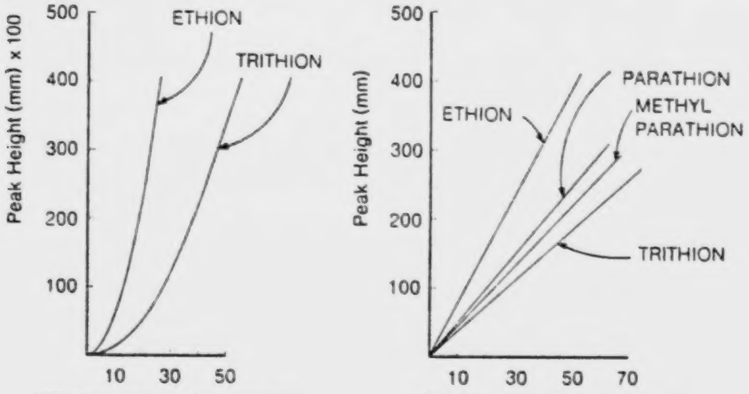
In the sulfur mode, the response is proportional to the square of the sulfur concentration. The detector is offered with a square root function circuit that linearizes the detector output, a necessity when electronic integration and automation in the sulfur mode are desired (Figure 1).

IV. PHOSPHORUS MODE:

When the detector is fitted with a 526 nm optical filter between the flame and the photomultiplier, it is highly selective for phosphorus, but large amounts of sulfur will give a response in this mode.

V. SULFUR MODE:

When the detector is fitted with a 394 nm filter, it becomes selective for sulfur. Sensitivity for sulfur is usually an order of magnitude less than for phosphorus.



FPD Response vs. Concentration
(Sulfur mode without square root function.)

FPD Response vs Concentration
(Sulfur mode with square root function.)

Fig. 1. Comparison of concentration vs response plots for FPD with and without sulfur-mode linearizer. Conditions: column, 183 cm x 4 mm glass, containing 3% OV-1 on high performance Chromosorb W; temperature, 200° C; carrier, nitrogen at 60 ml/minute.

GAS CHROMATOGRAPHY-FLAME PHOTOMETRIC

SAMPLE QUANTITATION AND INTERPRETATION

I. See Section 4,A,(4).

The priming mixture below is given in nanograms per microliter.

Ethyl Parathion	1.0	Malathion	1.0
Methyl Parathion	1.0	Ethion	1.0
Ronnel	0.5	Trithion	2.0
Diazinon	0.5		

Forty microliters of this mixture is injected. Do not inject with the same syringe used for routine sample injections.

II. Peak Height:

See Section 4,A,(5), I.

III. Peak Height x Width at Half Height:

See Section 4,A,(5), II.

NOTE: Both I and II presuppose that the absolute retention of standard and sample are the same.

IV. Triangulation or Integration:

See Section 4,A,(5), III.

V. Interpretation:

Because of the selectivity of the filters, interpretation is greatly simplified.

Identification of a thiophosphate can be accomplished in the following manner:

1. Retentions, relative to parathion (RRT_p), on a given column matched with a standard or matched against the RRT_p values given in Tables 2, 3 or 4.

2. Suspect compound in the correct Florisil elution.

3. Response on a selective detector.

4. Sulfur to phosphorus ratio matched against a standard.

TABLE I. RETENTION AND RESPONSE RATIOS, RELATIVE TO ETHYL PARATHION ON COLUMN OF 4% SE-30/6% QF-1

COMPOUND	RRT-P[1]	RPH-P[2]
TEPP	0.08	5.0
Dichlorvos	.10	5.0
Demeton-Thiono	.22	2.0
Naled	.28	0.02
Phorate	.28	4.0
Sulfotepp	.28	5.2
Diazinon	.35	2.5
Demeton-thiolo	.37	2.0
Dioxathion	.38	0.5
Disulfoton	.40	3.8
Diazinon-oxygen analog	.42	1.0
Dimethoate	.49	0.50
Monocrotophos	.54	0.08
Ronnel	.57	1.42
Ronnel-oxygen analog	.58	0.25
Chlorpyrifos	.68	1.4
Fenthion	.72	1.6
Methyl Parathion	.75	0.71
Malathion	.81	0.71
Methyl Parathion-oxygen analog	.83	0.10
Malathion-oxygen analog	.85	0.063
Fenitrothion	.85	0.80
Ethyl Parathion (reference)	1.00	1.00
Phosphamidon	1.02	0.16
Ethyl Parathion-oxygen analog	1.10	0.50
Merphos	1.23	0.35
DEF	1.25	0.80
Carbophenothion-oxygen analog	1.78	0.12
Ethion	1.83	0.71
Carbophenothion	1.90	0.36
Phenkapton	3.04	0.20
Fensulfothion	3.16	0.03
Imidan	3.91	0.02
EPN	3.95	0.134
Azinphos methyl	6.03	0.044
Coumaphos	11.84	0.20

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[1] Retention ratio, relative to ethyl parathion. Retention measurements from injection point.

[2] Peak height ratio, relative to ethyl parathion.

Table 2

4% SE-30/6% OV - 210
Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202	204	
0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	Dichlorvos
0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	TEPP
0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	Mevinphos
0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.21	Demeton Thiono
0.16	0.16	0.16	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.23	Thionazin
0.19	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.22	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	0.24	Ethoprop
0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	Phorate
0.21	0.22	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	Sulfotepp
0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.27	0.28	Neled
0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.26	0.28	0.29	Oxydemeton Methyl
0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.31	Diazinon
0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.35	Dioxathion
0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.37	Demeton Thiolo
0.30	0.31	0.31	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.37	Disulfoton
0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.40	0.40	0.40	Diazoxon
0.40	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	Dichlofenthion
0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	Dimethoate
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.52	0.52	0.52	Ronnel
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	Cyanox
0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.56	0.58	0.59	0.59	0.59	0.59	Ronnoxon
0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	0.61	0.61	Monocrotophos
0.58	0.59	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.62	0.62	0.62	0.62	Chlorpyrifos
0.57	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.62	0.62	0.62	Zytron
0.62	0.62	0.62	0.62	0.63	0.63	0.63	0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.65	0.65	0.65	Fenthion
0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70	Malaoxon
0.72	0.72	0.72	0.73	0.73	0.73	0.73	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	Methyl Parathion
0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79	0.78	0.78	0.78	0.78	0.78	Malathion
0.86	0.86	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.86	0.86	0.86	0.86	Fenitrothion
0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.90	0.89	0.89	0.88	0.88	0.88	0.87	Bromophos
0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	Methyl Paraoxon
0.93	0.93	0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	Phenthoate
0.91	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	Bromophos Ethyl
0.85	0.84	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.83	0.84	0.86	0.87	0.88	0.89	0.90	0.92	0.93	Schradan
0.96	0.96	0.96	0.96	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.98	Dicapthon
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	E. Parathion(Reference)
1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	1.00	1.01	1.01	1.01	1.01	1.01	1.01	Amidithion
1.02	1.02	1.02	1.02	1.02	1.02	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	Iodofenphos
1.11	1.11	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.08	Crufomate
1.18	1.18	1.17	1.17	1.17	1.16	1.16	1.15	1.15	1.15	1.14	1.14	1.13	1.13	1.13	1.12	1.12	1.11	DEF
1.19	1.18	1.18	1.17	1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.14	1.14	1.13	1.13	1.12	1.12	1.12	Phosphamidon
1.18	1.17	1.17	1.17	1.17	1.16	1.16	1.16	1.15	1.15	1.15	1.14	1.14	1.14	1.13	1.13	1.13	1.12	Folex
1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	1.17	1.16	Ethyl Paraoxon
1.23	1.22	1.22	1.22	1.22	1.22	1.21	1.21	1.21	1.21	1.20	1.20	1.20	1.20	1.19	1.19	1.19	1.19	Methidathion
1.37	1.36	1.36	1.35	1.35	1.34	1.33	1.33	1.32	1.32	1.31	1.31	1.30	1.30	1.29	1.29	1.28	1.28	Tetrachlorvinphos
1.87	1.85	1.84	1.83	1.82	1.81	1.80	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.70	1.69	1.68	1.67	Ethion
1.89	1.88	1.87	1.86	1.85	1.84	1.83	1.82	1.80	1.79	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.71	Carbophenoxon
1.89	1.88	1.87	1.86	1.85	1.84	1.83	1.82	1.80	1.79	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.71	Carbophenothion
3.18	3.15	3.12	3.09	3.06	3.03	3.00	2.98	2.95	2.92	2.89	2.86	2.83	2.80	2.77	2.74	2.72	2.69	Phenkapton
3.96	3.92	3.87	3.83	3.79	3.75	3.70	3.66	3.62	3.58	3.53	3.49	3.44	3.41	3.36	3.32	3.28	3.23	Fensulfothion
4.65	4.60	4.55	4.50	4.45	4.40	4.35	4.31	4.26	4.21	4.16	4.11	4.06	4.01	3.96	3.91	3.87	3.82	Imidan
4.66	4.61	4.56	4.51	4.46	4.41	4.36	4.31	4.26	4.21	4.16	4.11	4.06	4.00	3.95	3.90	3.85	3.80	EPN
5.67	5.63	5.59	5.55	5.51	5.47	5.42	5.38	5.34	5.30	5.26	5.21	5.17	5.13	5.09	5.05	5.01	4.96	Famphur
7.48	7.38	7.27	7.17	7.06	6.96	6.85	6.75	6.64	6.54	6.43	6.33	6.22	6.12	6.01	5.91	5.80	5.70	Azinphos Ethyl
7.53	7.42	7.32	7.21	7.10	6.99	6.89	6.78	6.67	6.57	6.46	6.35	6.24	6.14	6.03	5.92	5.81	5.71	Azinphos Methyl
17.4	17.0	16.7	16.3	16.0	15.6	15.3	15.0	14.6	14.3	13.9	13.6	13.2	12.9	12.6	12.2	11.9	11.5	Coumaphos
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to parathion, of 54 organophosphorous pesticides on a column of 4% SE-30/6% OV-210 at temperatures from 170 to 204°C; support of Gas Chrom-Q, 80/100 mesh; flame photometric detector, 5260 A°filter; all absolute retentions measured from injection point. Arrow indicates optimum operating temperature with carrier flow set at 75 ml per minute.

Table 3

10 % OV-210
Column Temperature , °C.

170		174		178		182		186		190		194		198	↓	202	204	
0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	TEPP
0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	Dichlorvos
0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	Demeton Thiono
0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.19	Mevinphos
0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.19	Thionazin
0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	Phorate
0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	Ethoprop
0.17	0.17	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21	Diazinon
0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.22	0.22	0.23	Sulfotepp
0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	Naled
0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	Oxydemeton Methyl
0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	Disulfoton
0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.31	Dioxathion
0.29	0.29	0.29	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.32	0.32	Demeton thiolo
0.29	0.29	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.34	0.35	Dichlofenthion
0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.36	Diazoxon
0.34	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.39	0.40	Ronnel
0.40	0.40	0.41	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.42	0.43	0.43	0.43	0.43	0.43	0.43	Chlorpyrifos
0.43	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	Zytron
0.47	0.47	0.47	0.47	0.47	0.47	0.48	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.50	0.50	Bromophos
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	Fenthion
0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	Dimethoate
0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	Ronnoxon
0.53	0.53	0.54	0.54	0.54	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.58	0.59	0.59	Cyanox
0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	0.61	Bromophos ethyl
0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	0.63	Monocrotophos
0.75	0.75	0.75	0.74	0.74	0.73	0.73	0.73	0.72	0.72	0.71	0.71	0.71	0.70	0.70	0.70	0.69	0.69	Malathion
0.69	0.69	0.69	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.71	0.71	0.71	0.71	0.71	Iodofenphos
0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.75	0.74	DEF
0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.75	0.75	0.75	Phenthoate
0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	Folex
0.74	0.74	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	0.77	0.77	0.77	0.77	0.78	0.78	0.78	0.79	Methyl parathion
0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.81	Schradan
0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.86	0.86	0.86	Fenitrothion
1.02	1.01	1.01	1.00	0.99	0.99	0.98	0.97	0.96	0.96	0.95	0.94	0.94	0.93	0.92	0.92	0.91	0.90	Malaoxon
0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.95	Dicapthon
1.02	1.02	1.02	1.01	1.01	1.00	1.00	1.00	0.99	0.99	0.99	0.98	0.98	0.98	0.97	0.97	0.97	0.96	Crufomate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Parathion **(Reference)**
1.04	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	0.99	Methyl paraoxon
1.03	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.01	1.00	Amidithion
1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.12	1.12	1.12	1.12	1.12	1.12	1.12	Methidathion
1.22	1.22	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.13	1.12	Tetrachlorvinphos
1.27	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	1.20	1.19	Carbophenothion
1.38	1.37	1.36	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.29	1.28	1.27	1.26	1.26	1.25	1.24	Phosphamidon
1.42	1.41	1.40	1.39	1.38	1.37	1.37	1.36	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.29	1.28	1.27	Ethyl paraoxon
1.43	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.37	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.29	Ethion
1.64	1.63	1.62	1.62	1.61	1.60	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.53	1.52	1.51	1.50	1.49	Carbophenoxon
2.09	2.08	2.06	2.04	2.02	2.00	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	1.80	1.79	1.77	Phenkapton
4.26	4.21	4.16	4.11	4.06	4.01	3.97	3.92	3.87	3.82	3.77	3.72	3.68	3.63	3.58	3.53	3.48	3.43	Fensulfothion
4.37	4.32	4.27	4.22	4.18	4.13	4.08	4.03	3.98	3.93	3.88	3.83	3.78	3.73	3.68	3.63	3.58	3.53	EPN
4.65	4.59	4.54	4.48	4.43	4.37	4.32	4.26	4.21	4.15	4.10	4.04	3.99	3.94	3.88	3.86	3.77	3.72	Imidan
6.98	6.88	6.77	6.66	6.55	6.44	6.33	6.22	6.11	6.00	5.89	5.78	5.67	5.56	5.45	5.34	5.24	5.13	Azinphos methyl
6.86	6.76	6.66	6.56	6.47	6.37	6.27	6.17	6.08	5.98	5.88	5.79	5.69	5.59	5.49	5.40	5.30	5.20	Famphur
7.01	6.90	6.79	6.69	6.58	6.47	6.36	6.25	6.14	6.03	5.92	5.81	5.70	5.59	5.48	5.37	5.26	5.15	Azinphos ethyl
19.1	18.7	18.3	17.9	17.5	17.1	16.7	16.3	15.9	15.5	15.1	14.7	14.3	13.9	13.5	13.1	12.7	12.3	Coumaphos
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to ethyl parathion, of 54 organophosphorous pesticides on a column of 10% OV-210 at temperatures from 170 to 204°C; support of Gas Chrom Q, 100/120 mesh; flame photometric detector, 5260 A° filter; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml per minute.

Table 4

1.5% OV-17/1.95% OV-210
Column Temperature, °C.

170		174		178		182		186		190		194		198	↓	202	204	
0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.09	TEPP
0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.09	0.09	0.09	0.10	0.10	0.10	0.11	0.11	0.11	0.12	Dichlorvos
0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20	0.21	0.21	Mevinphos
0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.21	0.21	0.21	0.22	Demeton thiono
0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.25	0.25	0.25	Thionazin
0.20	0.20	0.20	0.21	0.21	0.22	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.26	0.26	0.26	Ethoprop
0.23	0.23	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.29	0.29	0.29	0.30	Phorate
0.24	0.25	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.30	Sulfotepp
0.26	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32	0.32	0.33	0.33	Oxydemeton methyl
0.32	0.32	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.37	Diazinon
0.31	0.31	0.32	0.32	0.33	0.33	0.34	0.34	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.38	0.38	0.39	Naled
0.34	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.39	0.39	Demeton thiclo
0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.42	0.42	0.42	0.43	Disulfoton
0.37	0.38	0.38	0.39	0.39	0.39	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.43	0.43	0.43	0.44	0.44	Dioxathion
0.38	0.39	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.43	0.43	0.44	0.44	Diazoxon
0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.49	0.50	Dichlofenthion
0.51	0.51	0.51	0.52	0.52	0.53	0.53	0.53	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.57	Cyanox
0.53	0.53	0.53	0.54	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.58	Dimethoate
0.55	0.55	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	0.61	0.61	0.62	Ronnel
0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	0.61	0.61	0.62	0.62	0.63	0.63	0.63	Ronnoxon
0.78	0.77	0.76	0.76	0.75	0.74	0.73	0.73	0.72	0.72	0.71	0.70	0.69	0.68	0.68	0.67	0.66	0.65	Monocrotophos
0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.71	Zytron
0.74	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	0.76	0.76	Chlorpyrifos
0.74	0.74	0.74	0.75	0.75	0.75	0.76	0.76	0.77	0.77	0.77	0.78	0.78	0.78	0.79	0.79	0.79	0.80	Methyl Parathion
0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	Methyl Paraoxon
0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.93	0.89	0.89	0.89	0.88	0.88	0.87	0.87	0.87	0.86	Malaoxon
0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	0.89	0.89	0.89	0.89	Malathion
0.85	0.85	0.86	0.86	0.86	0.86	0.87	0.87	0.87	0.87	0.88	0.89	0.88	0.88	0.89	0.89	0.89	0.90	Bromophos
0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	0.90	Fenthion
0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.92	0.92	0.92	0.92	0.92	0.92	Fenitrothion
1.02	1.01	1.01	1.00	1.00	1.00	0.99	0.99	0.98	0.98	0.98	0.97	0.97	0.96	0.96	0.96	0.95	0.95	Phosphamidon
1.25	1.23	1.21	1.19	1.18	1.16	1.14	1.13	1.11	1.09	1.07	1.06	1.04	1.02	1.00	0.99	0.97	0.95	Schradon
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Parathion(Reference)
1.06	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.04	1.04	1.04	1.04	1.04	1.04	Ethyl Paraoxon
1.05	1.05	1.05	1.05	1.06	1.06	1.06	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.04	1.04	1.04	1.03	Dicapthon
1.12	1.12	1.12	1.11	1.11	1.11	1.11	1.11	1.11	1.10	1.10	1.10	1.10	1.10	1.09	1.09	1.09	1.09	Bromophos Ethyl
1.23	1.22	1.22	1.22	1.21	1.20	1.20	1.19	1.19	1.19	1.19	1.19	1.20	1.20	1.20	1.20	1.20	1.20	Amidithion
1.40	1.39	1.38	1.38	1.37	1.36	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	Crufomate
1.36	1.35	1.35	1.34	1.34	1.33	1.33	1.32	1.32	1.31	1.31	1.30	1.30	1.29	1.29	1.28	1.27	1.27	Phenthoate
1.51	1.50	1.49	1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	Folex
1.51	1.50	1.49	1.49	1.48	1.47	1.46	1.45	1.44	1.44	1.43	1.42	1.41	1.41	1.40	1.39	1.38	1.37	DEF
1.57	1.57	1.56	1.56	1.56	1.55	1.54	1.54	1.53	1.53	1.53	1.52	1.52	1.51	1.51	1.50	1.50	1.49	Iodofenphos
1.72	1.71	1.70	1.69	1.68	1.67	1.66	1.65	1.65	1.64	1.63	1.62	1.61	1.60	1.59	1.58	1.57	1.56	Tetrachlorvinphos
1.74	1.73	1.73	1.72	1.72	1.71	1.71	1.70	1.70	1.69	1.69	1.68	1.68	1.67	1.67	1.66	1.65	1.65	Methidathion
2.69	2.67	2.64	2.62	2.59	2.57	2.54	2.52	2.49	2.46	2.45	2.42	2.40	2.37	2.35	2.32	2.30	2.27	Carbophenoxon
2.88	2.85	2.82	2.79	2.76	2.73	2.70	2.67	2.64	2.61	2.58	2.55	2.52	2.49	2.46	2.43	2.40	2.37	Ethion
2.99	2.97	2.94	2.92	2.89	2.87	2.84	2.82	2.79	2.77	2.75	2.72	2.70	2.67	2.66	2.62	2.60	2.57	Carbophenothion
4.65	4.60	4.56	4.51	4.46	4.42	4.37	4.32	4.27	4.23	4.18	4.13	4.08	4.04	3.99	3.94	3.89	3.85	Fensulfothion
5.57	5.50	5.42	5.35	5.27	5.20	5.12	5.05	4.97	4.90	4.82	4.75	4.67	4.60	4.52	4.45	4.38	4.30	Phenkapton
6.07	5.99	5.90	5.82	5.74	5.65	5.57	5.49	5.40	5.32	5.24	5.15	5.07	4.99	4.90	4.82	4.74	4.65	Famphur
6.63	6.53	6.44	6.34	6.25	6.16	6.06	5.97	5.88	5.78	5.69	5.59	5.50	5.41	5.31	5.22	5.12	5.03	EPN
7.95	7.84	7.72	7.61	7.50	7.38	7.27	7.15	7.04	6.93	6.81	6.70	6.58	6.50	6.36	6.24	6.13	6.01	Imidan
10.9	10.7	10.6	10.4	10.3	10.1	10.0	9.8	9.7	9.5	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	Azinphos Methyl
14.4	14.1	13.9	13.6	13.3	13.0	12.8	12.5	12.2	11.9	11.7	11.4	11.1	10.9	10.6	10.3	10.1	9.8	Azinphos ethyl
22.2	21.8	21.3	20.9	20.4	20.0	19.5	19.1	18.6	18.2	17.2	17.3	16.8	16.4	15.9	15.5	15.0	14.6	Coumaphos
170		174		178		182		186		190		194		198		202	204	

Retention ratios, relative to ethyl p[illegible]nion, of 54 organophosphorous pesticides on a column of 1.5% OV-17/1.95% OV-210 at temperatures from 170 to 204°C; column support of Gas Chrom-Q, 100/120 mesh; flame photometric detector, 5260 A° filter; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml per minute.

FIGURE 1

Carbowax Tube Section

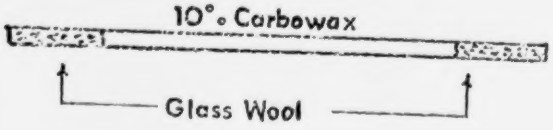

10% Carbowax
Glass Wool

FIGURE 2

Cutaway View of Column with Carbowax Assembly

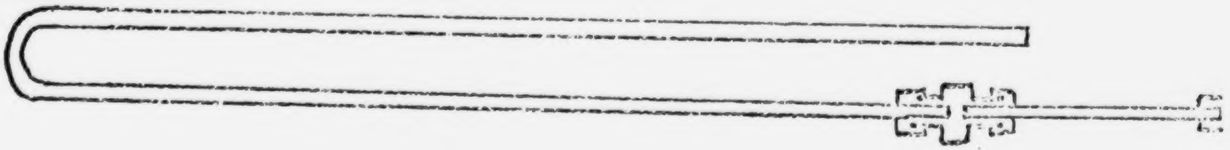


FIGURE 3

Chromatograms of a mixture of 7 organophosphorous pesticides on an untreated column of 4% SE-30/6% QF-1 (Fig.1), and on the same column treated with Carbowax (Fig.2)

Column: 4% SE-30/6% QF-1; amps, full scale 0.8 x10 -8 ; voltage 850 v.

OPERATING PARAMETERS

TEMP., °C.		FLOW RATES, ml/min.	
Column	200	Carrier	60
Inlet	225	Vent	60
Detector	195	Oxygen	30
Transf. line	235	Hydrogen	180
Vent	235	Air	40

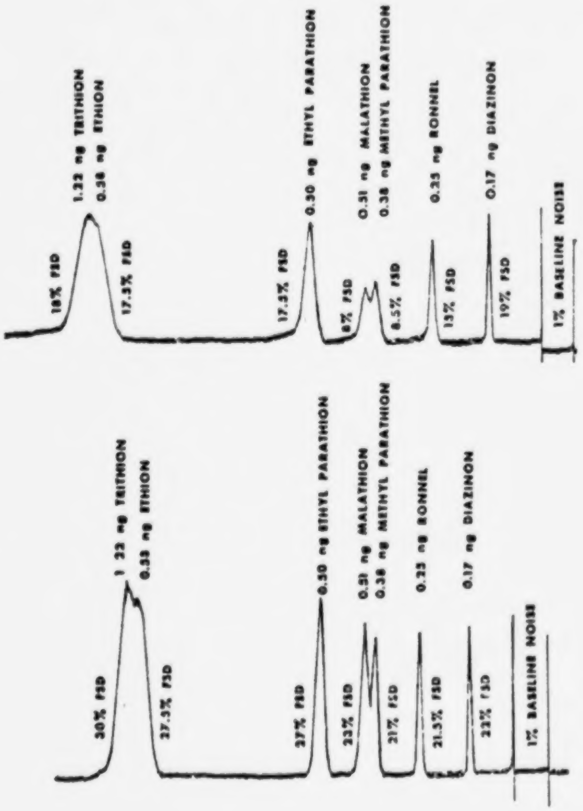
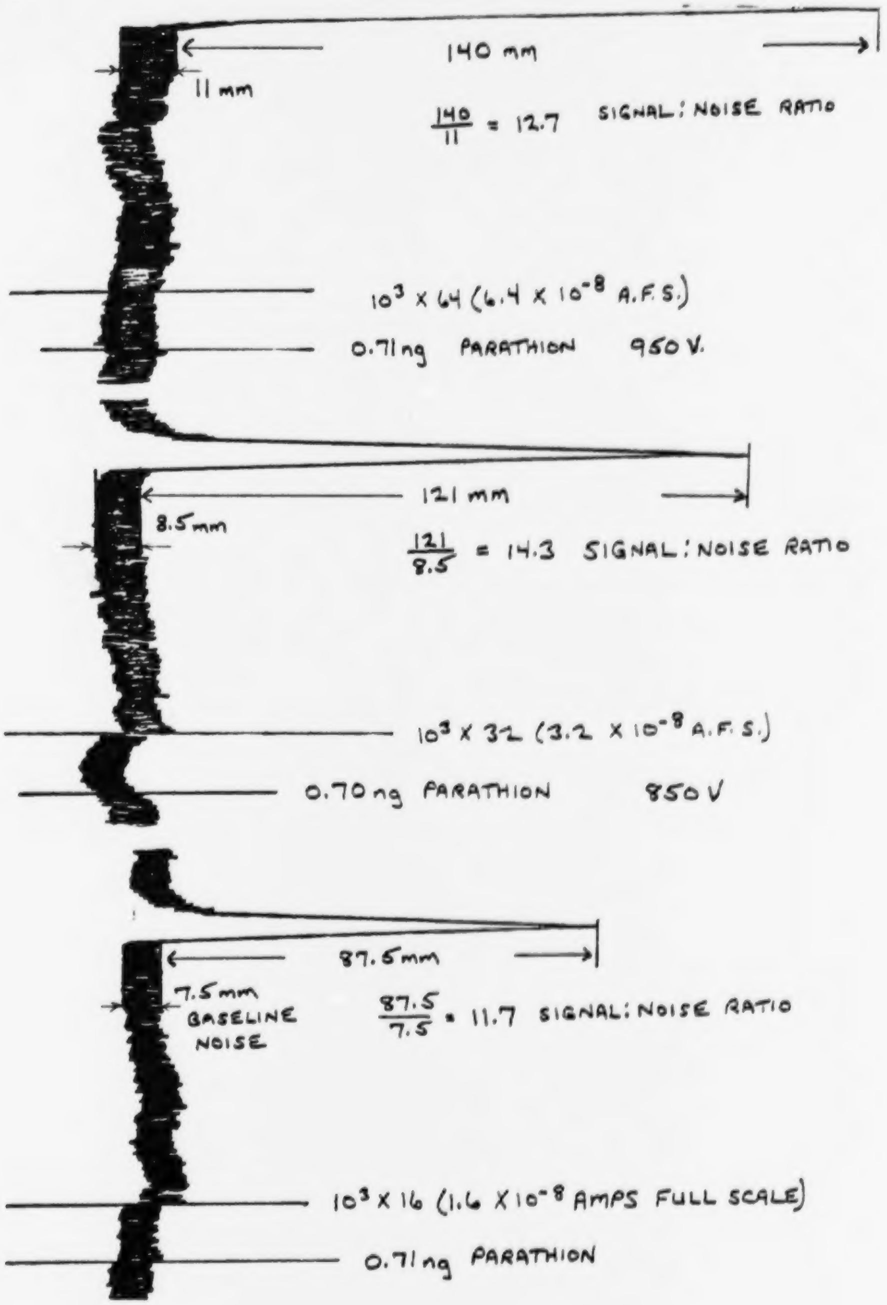


FIGURE 4. Calculations of Signal/Noise Ratio



GAS CHROMATOGRAPHY - HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

INSTRUMENT

I. INTRODUCTION:

The Hall electrolytic conductivity detector (HECD) can be operated in the reductive mode selective for nitrogen- or halogen-containing compounds or in the oxidative mode selective for sulfur detection. Most applications to date have been for organochlorine and organonitrogen compounds. The selectivity of the detector allows its use for obtaining confirmatory evidence for residues tentatively identified by electron capture GC, while the sensitivity is adequate for quantitations at sub-ppm levels.

Effluent from the gas chromatograph is pyrolyzed in a quartz combustion tube in the presence of specified gases and sometimes catalysts. During pyrolysis, specific elements present in the organic pesticides form soluble electrolytes that are then combined with deionized liquid in a gas-liquid contactor. The electrical conductivity of the liquid is continuously measured. Only those combustion products that are readily soluble and ionized in the liquid change its electrical conductivity and produce a response on the recorder.

Most of the material in this section applies specifically to the Tracor Model 700 Hall detector connected to a conventional gas chromatograph such as the Tracor MT 222, or equivalent. The Model 700 monitors the electrical conductivity of the liquid utilizing an AC bridge circuit and auxiliary recorder. The newer Model 700A detector is similar in principle but is engineered exclusively for use with the Tracor Model 560 digital processor controlled gas chromatograph. The 700A features more precise flow regulation, a microreactor furnace, lower dead volume, improved scrubbers, automatic solvent venting, and a new differential conductivity cell design combined with a pulse cell excitation system for improved detection specificity and sensitivity and baseline stability.

II. FLOW SYSTEM:

See page 1 of Section 4,A,(1). Hydrogen is the combustion gas for both chlorine and nitrogen detection in the reductive mode. Air is the reactor gas for sulfur compounds in the oxidative mode. Helium only (preferably ultra pure) is the recommended carrier gas for operation in the nitrogen mode. Nitrogen carrier in this mode is unsatisfactory because a small percentage of nitrogen is converted to ammonia causing a high background and low sensitivity. Helium may also be used in the chloride (reductive) mode, but best results are obtained only with the ultra pure grade. Nitrogen or helium may be used as carrier gas in the sulfur mode. In all cases, metal diaphragm pressure regulators should be used with helium to prevent contamination of the carrier gas with air. A Go-Getter gas purifier (General Electric, Schenectady, NY, distributed by Alltech Associates, Inc., Arlington Heights, IL) can be used to ensure removal of impurities from helium. Hydrogen must be free of oxygen to be suitable. Electrolytic generators constructed with a palladium diffusion membrane have been successfully used with the HECD.

The HECD is extremely sensitive to carrier gas leaks, with generally are manifested as wandering baseline and noise.

III. DETECTOR:

See Section 4,C,(3). For connection of the detector to the chromatograph and operation of the system, see Sections II and III of the Model 700 Operation and Service Manual 115008A.

IV. ELECTROMETER:

See Section 4,A,(1), III.

The HECD does not require as high a voltage as the electron capture and FPD detectors. The power supply for the HECD shares a printed circuit board with the AC bridge. The function of the power supply is to provide + 15 V regulated.

V. TEMPERATURE PROGRAMMER:

See Section 4,A,(1), IV.

VI. PYROMETER:

See Section 4,A,(1), V.

VII. MISCELLANEOUS:

See Section 4,A,(1), VI.

A heated transfer line carries the GC column effluent to the combustion tube, and this line plays a critical role in successful operation of the detector. Care must be taken to ensure that all transfer line areas are sufficiently hot to prevent analyte loss and/or tailing.

Use of septa coated with polyimide (Pursep-P, L. C. Co., Inc., Schaumburg, IL) has been reported to reduce background noise during HECD operation (FDA PAM, Section 315.42, 6).

GAS CHROMATOGRAPHY - HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

COLUMNS

I. SPECIFICATIONS:

See Section 4,A,(2), page 1.

II. COLUMN SELECTION:

Although normal pesticide column packings (Section 4,A,(2)) have most often been used with the HECD, it is best to select very stable columns because acid products resulting from the bleed of halogenated liquid phases, such as OV-210, XE-60, or QF-1, can produce inordinately high noise levels in the Cl-mode and scrubber depletion in the N-mode. For this reason, surface-bonded Carbowax 20M columns (Section 4,A,(7)), uncoated or coated with a low loading of a stable liquid phase, are especially recommended for analyses with the HECD (Section 12,A). Columns containing 3% OV-1, 3% and 5% OV-101, 3% STAP, 5% Carbowax 20M, and wall-coated OV-101 (capillary column) have also been successfully used.

The use of 1.8 m (6 ft.) x 2 mm i.d. GC columns rather than 4 mm i.d. has been recommended in the FDA PAM (Section 315.41 (3)). The smaller column requires only 30-40 ml/minute carrier gas flow to produce the same chromatogram from the larger column with 100-120 ml/minute flow, leading to increased detector sensitivity and reduced system back pressure. The smaller column may not, however, tolerate a large number of relatively "dirty" samples.

III. PACKING THE COLUMN:

See Section 4,A,(2), III.

IV. COLUMN CONDITIONING:

See Sections 4,A,(2), IV and 4,A,(7), II.

V. COLUMN EVALUATION:

See Section 4,A,(2), V for general evaluation guidelines.

Figures 1 and 2 show typical sensitivities attainable for pesticides with the nitrogen and chloride modes of the Model 700

HECD using the following operating conditions:

Column	1.8 m (6 ft.) x 4 mm (¼ in.), 3% OV-1
Column oven	200°C
Inlet	200°C
Transfer line	275°C
Helium carrier	50 ml/minute
Hydrogen reaction gas	50 ml/minute
Electrolyte	*n*-propanol-deionized water (1:1 v/v) *or* 100% methanol for the chlorine mode
Electrolyte flow	0.8 ml/minute
Furnace temperature	850°C
Background level	<1% FSD at 8 x 10
Noise	5% peak to peak at 1 x 10
Chart speed	0.5 in./minute

Figure 3 shows the sensitivity for simazine with a 5% OV-101 column with the nitrogen mode Model 700 HECD. The operating conditions are listed on the figures.

The chromatograms shown in the figures in Section 4,A,(7) indicate sensitivities and retention times to be expected with the Model 700 HECD and Carbowax 20M columns.

Figures 4, 5, and 6 present chromatograms for pesticides in the halide, nitrogen, and sulfur modes, respectively, with the Model 700 HECD detector. The columns were 3% OV-101 for chlorinated and phosphorus pesticides and 3% STAP for triazines. In general, 1-2 orders of magnitude better sensitivity can be expected for the Model 700 A HECD compared to the Model 700.

If columns are properly prepared and conditioned and operating conditions are optimized, the analyst should be able to reproduce or improve the sensitivities and chromatograms illustrated in these sections for pesticide standards.

Relative retention times and responses for numerous pesticides on 5% or 10% DC-200 columns with the nitrogen-mode HECD are listed in the FDA PAM, Table 335-A. A chromatogram of 2.5-5 ng of seven OCl insecticides on a 5% OV-101 column with the HECD in the halogen mode is given in the FDA PAM, Figure 335-B.

VI. MAINTENANCE AND USE OF COLUMNS:

See Sections 4,A,(2), VI and 4,A,(7), II.

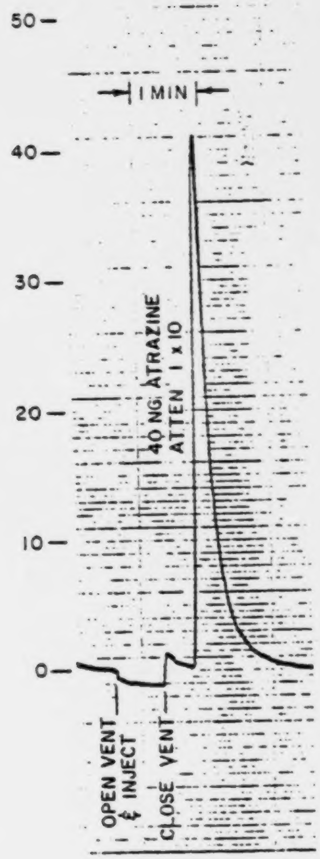


Fig. 1. Typical chromatogram Hall electrolytic conductivity detector in the nitrogen mode.

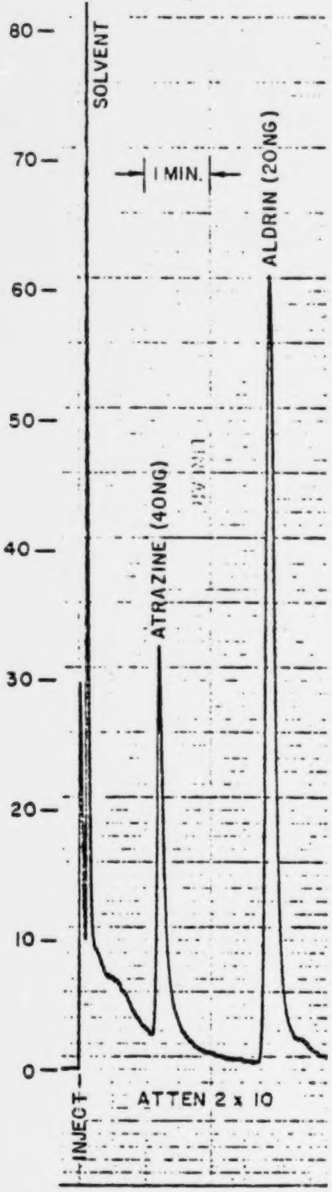
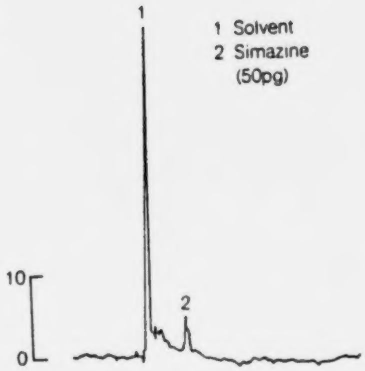
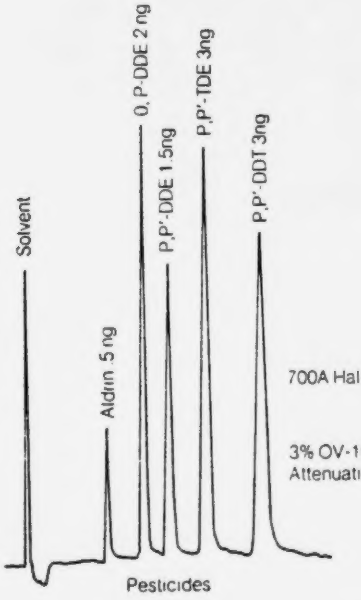


Fig. 2. Typical chromatogram Hall electrolytic conductivity detector in the chloride mode.



Conditions
Detector: Model 700 Hall Detector
Mode: Nitrogen with Ni Catalyst
Reaction Gas: H_2, 40 cc/min
Furnace Temp: 800° C
Solvent: 1:4 IPA/water
Column: 6' Glass; 5% OV-101 on Gas
Chrom Q
Sensitivity: 10 x 1
Col. Temp: 200° C

Fig. 3. Chromatography of simazine.



700A Halide Mode

3% OV-101 Column @200°C
Attenuation 10 x 4

Fig. 4. Chromatogram of chlorinated pesticides.

CONDITIONS

GC: TRACOR MODEL 560
Column: 3% STAP on 80-100 CWHP,
6' x 2mm ID Glass
Column temp.: 195°C
Carrier: 30 ml/min, He
Injector temp.: 210°C
Detector: 700A HECD
Detector temp.: 225°C
Reactor temp.: 800°C
Reaction gas: 80 ml/min, H_2

COMPOUNDS

1 Propazine
2 Atrazine
3 Simazine
4 Prometryne
} 4 ng each

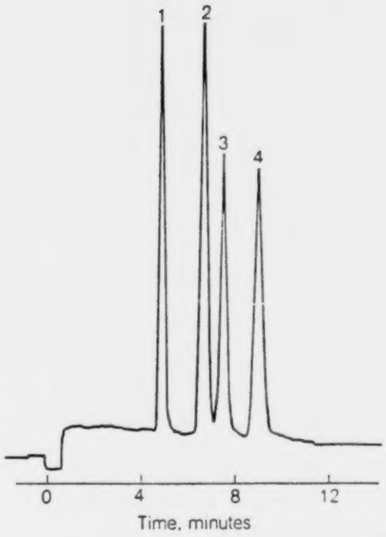


Fig. 5. Chromatogram of nitrogen herbicides.

CONDITIONS

GC: TRACOR MODEL 560
Column: 3% OV-101 on 80-100 CWHP
6' x 2mm ID Glass
Column temp.: 190°C
Carrier: 25 ml/min, He
Injector temp.: 210°C
Detector: 700A HECD
Detector temp.: 225°C
Reactor temp : 850°C
Reaction gas: 100 ml/min, air

Compounds
1 Thimet = 1.8 ng
2 Malathion
3 Parathion
4 Captan
5 Ethion
6 Trithion
} 3.6 ng each

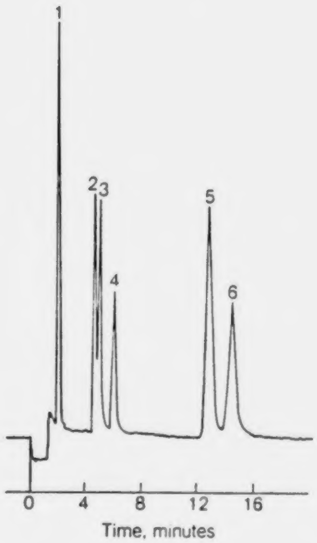


Fig. 6. Chromatogram of sulfur pesticides.

GAS CHROMATOGRAPHY - HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

DETECTOR

I. OPERATING PARAMETERS:

See Section 4, C, (2), V for a list of typical but not critical operating conditions for the HECD. The column oven temperature will, of course, affect elution time in the usual way. The inlet and transfer line temperatures may be decreased by as much as 50°C with little effect. The helium carrier flow rate can be increased or decreased 5-10% without major effect (except for the expected change in elution time). The hydrogen reaction gas flow rate can be set anywhere in the range of 40-50 ml/minute; above this rate, response may decrease. The concentration of n-propanol will not drastically affect results within the range of 40-60%. The peak height is inversely related (but probably not linearly) to the electrolyte flow into the gas liquid contactor. However, at flow rates less than 0.3-0.4 ml/minute, background noise becomes appreciable. The conversions in the furnace proceed well at temperatures of 820-850°C. Above this temperature, response increases for some compounds and decreases for others. Certain classes of compounds may be selectively screened for at a given temperature. For example, at 600°C, reduced response is noted for chlorinated pesticides, but PCBs elicit no response at all. Therefore, theoretically the pesticides could be quantitated at this temperature, and the PCBs could be determined by difference after determining total chlorine response at 830°C.

II. MODES OF DETECTION AND SELECTIVITY:

The basic components of the electrolytic conductivity detector are displayed in Figure 1. The chromatographed sample is converted to the monitored species by oxidative or reductive pyrolysis in the high temperature furnace. The reaction products are swept into a gas-liquid contactor where they are mixed with a conductivity solvent. The liquid phase is separated from insoluable gases in a gas-liquid separator and then passed through a conductivity cell (Figure 2).

The Hall detector, which is a miniaturized version of the original Coulson electrolytic conductivity detector, possesses significantly higher sensitivity. This is the result of modifications in cell design and geometry leading to decreased detector dead volume, and improved electronic measuring of electrolytic conductivity. The selectivity of the HECD in the N-, Cl-, and S-modes vs hydrocarbons is equal to 10^6 or greater in all cases.

Nitrogen Detection - Organic nitrogen-containing pesticides are converted to ammonia at 800-900°C with a nickel wire catalyst and hydrogen reaction gas according to the following equation:

$$\text{R-CN} \xrightarrow{\text{Ni, H}_2} \text{NH}_3 + \text{Lower Alkanes}$$

The increase in conductivity due to the formation of ammonium hydroxide is the basis of response.

$$\text{NH}_3 + \text{H}_2\text{O} \rightleftarrows \text{NH}_4^{+} + \text{OH}^{-}$$

Selectivity to nitrogen compounds is based on the conversion of potentially interfering substances to reaction products that either produce little electrolytic conductivity or can be easily removed with a post-pyrolysis scrubber prior to the cell. Specifically, pesticides containing halogens and sulfur are converted to HX and H_2S (and/or H_2SO_3, H_2SO_4), respectively, all of which are removed by inserting a nitrogen mode scrubber tube containing, e.g., strontium hydroxide on Fiberfrax or quartz wool, inside the exit end of the combustion tube. Compounds containing oxygen are converted to water (no response). Lower alkanes (primarily CH_4) are also produced in all cases, but these have low solubilities in the conductivity solvent and are not ionized when dissolved (no response). Thus, ammonia is the only product from common organic compounds that gives a response, thereby explaining the high specificity to nitrogen compounds.

The selectivity due to the scrubber is illustrated by Figure 3. Chromatogram A shows the separation of two representative nitrogen-containing compounds, atrazine and caffeine, at the 10 ng level, along with four chlorinated insecticides at the 100 ng level. Note that the atrazine and caffeine peaks (numbered 1 and 3) are nearly obscured by the chlorinated compounds. Chromatogram B shows the same mixture run under identical conditions except that the strontium hydroxide scrubber was inserted in the furnace tube to remove the interfacing halogen peaks. For the maximum selectivity, the scrubber tube must be maintained in efficient condition.

In practice, sample extracts with microgram quantities of sulfonated compounds will cause significant detector response. Sample impurities containing nitrogen can also be present in sample extracts and will cause unwanted response.

Chlorine Detection - In the reductive mode, ammonia will not form from nitrogen-containing compounds unless the nickel catalyst is present. Therefore, chlorinated pesticides can be selectively detected as HCl using an empty pyrolysis tube and hydrogen reactor gas. As in the nitrogen mode, the products H_2S and CH_4 will cause little or no response.

Any oxygen in the system, introduced either as a carrier or combustion gas impurity or from the sample itslef (e.g., a sulfoxide or sulfone group), will form acid complexes that cause considerable response. Small amounts of solvents containing oxygen, such as ethyl acetate, can render the detector inoperable. Selectivity to chlorine, therefore, depends on the high purity of the carrier and combustion gases and solvents, efficiency of oxygen traps in the gas lines, and cleanup of sample matrices to remove impurities containing groups such as S-O. Bleed of GC liquid phases containing halogenated compounds can also cause interferences.

Adequate cleanup of many samples for determination with the HECD in the nitrogen and chloride modes can be obtained by use of the modified MOG procedure described in Section 5,A,(1). Additional cleanup of fatty samples, if required, can be achieved by gel permeation chromatograpny (Sections 12,A and 5,B).

Sulfur Detection - When used in the oxidative mode, the eluting compounds are combined with air in an empty pyrolysis tube, forming different reaction products. Chlorine-containing compounds again produce HX, which is removed by a silver nitrate scrubber. Nitrogen-containing compounds form N_2 or NO_2 in very low yield (no response). Hydrocarbons produce CO, CO_2, and H_2O (no response). Sulfur groups produce SO_2 and SO_3, which dissolve in the electrolyte to yield ionized compounds providing the selective response of the detector.

Almost no use has been made of the sulfur mode of the HECD for practical residue analyses. The reportedly lower selectivity and sensitivity compared to the chloride and nitrogen modes will apparently limit the potential of the sulfur mode for pesticide determinations.

III. BACKGROUND SIGNAL CHECK:

1. Set attenuator to infinity (INF) position and zero recorder.

2. Set the attenuator to 8, the conductivity range to 10, and the zero suppression switch to off.

3. Normally, with a well stabilized system, the background should be less than 10% of full scale. A background significantly higher than this may result in negative peaks. High background levels are normally caused by column bleed, contamination of the reaction or carrier gas, leaks or contamination elsewhere in the system, or incompletely conditioned furnace tubes. In the latter event, the background level will gradually decrease over a period of time and

will normally reach acceptable to excellent levels in a day or so (background less than 5% and noise less than ½% at 10 x 8). When the instrument is first set up, typical background and noise levels may be as high as 60% and 3%, respectively, at an attenuation of 10 x 8.

4. Activate zero suppression switch and adjust coarse zero and fine zero controls to suppress background signal to zero.

IV. SENSITIVITY:

See the chromatograms in Section 4,C,(2) for typical sensitivities obtained for the HECD in various modes with different GC columns. In general, the HECD is less sensitive than electron capture or N-P detectors, due, in part, to the relatively large dead volume of the detector.

Chlorine Detection - the 2 mm i.d. furnace tube is recommeneded over the 4 mm tube for chlorine detection since better chromatography with less peak broadening results from its use. Best sensitivity is obtained when the conductivity solvent is neutral or slightly acid. Under ideal conditions, a response of ½ FSD to 1 ng of heptachlor epoxide can be obtained. For reliable, noise-free operation, ½ FSD for 5-10 ng heptachlor epoxide should readily be obtainable (FDA PAM, Section 315.32 (1)).

Nitrogen Detection - The 4 mm i.d. furnace tube is preferable because contact time between the sample and the Ni catalyst is increased and conversion to ammonia is enhanced. A larger amount of scrubber can also be placed in the larger tube. Conductivity solvent should be neutral or slightly basic for optimum sensitivity. Negative peaks can occur if pH is less than 7. As little as 0.1-0.5 ng of nitrogen (corresponding to ca. 1.5-7.5 ng carbaryl or ca. 0.3-1.5 ng atrazine) can produce ½ FSD response during optimized operation, although 5-10 times more nitrogen may be required for the same response during routine operation (FDA PAM, Section 315.32 (2)).

The Tracor company reports the following sensitivity specifications for the HECD:

Model 700	Model 700A
below 0.01 ng N, CL, or S	halogen - 5×10^{-13} g Cl/second
with noise $<1\%$ at 1 x 10	nitrogen - $2\text{-}4 \times 10^{-12}$ g N/second
attenuation	sulfur - $1\text{-}2 \times 10^{-12}$ g S/second

A rapid decrease in response can result from injection of lipid extracts that have not been subject to rigorous cleanup.

V. LINEARITY:

Linearity of response is reported by the manufacturer to be 10^6 for Cl (Fig. 3), 10^4 for N, and $>10^4$ for S. The FPD is not linear for sulfur unless electronically compensated. Linearity at low concentrations depends on the cleanliness of the system and the correct pH of the conductivity solvent as determined by the condition of the ion exchange resin. The upper end of the linear range for nitrogen is determined by the amount and condition of the nickel catalyst as well as its position in the furnace (FDA PAM, Section 315.33).

VI. EQUIPMENT AND REAGENTS:

1. A potential source of interference is unsuspected reactions in the furnace caused by contamination. For this reason, it is recommended that Vespel (DuPont), graphite, or glass filled Teflon ferrules be used throughout the system, together with the most stable column materials available. Silicone O-rings may be used in place of ferrules for lower temperature work, but a certain amount of bleed will be encountered until they become well conditioned.

   NOTE: Vespel ferrules sometimes stick in the fitting after high temperature operation and cannot be removed easily by pulling or twisting the quartz furance tube. The best procedure is to back off the Swagelok nut completely and grasp the exposed portion of the ferrule with tweezers as close to the metal fitting as possible and twist slightly until it is dislodged. To avoid this problem, glass filled Teflon ferrules (Tracor Part No. 76458-0012) can be used with no sacrifice in performance up to 275°C.

2. For convenience, the type "T" solvent vent of the Model 700 can be replaced with a four port high temperature valve (e.g., Model CV-4-HTA, Valco Instruments Co., Houston, TX). This valve vents solvent while still maintaining gas flow through the detector from the column in use. In addition, effluent from either of two different GC columns can be readily delivered to the pyrolysis furnace.

   To operate, the valve should be opened immediately before a sample injection, left open for a time period sufficient to vent all the solvent, and closed before the first sample peak begins to elute. For best results, it is important that the venting time be repeated, as closely as possible, for each successive injection. Under normal operating conditions, 30 seconds vent time is usually adequate for most solvents. If 10 microliters or more is injected, more time may be required.

3. The needle valve solvent flow controller to the gas-liquid contactor can be replaced with a more exact 10-turn calibrated control (e.g., stainless steel Nupro fine metering valve with vernier, catalog No. M-ZMA) for more stability and reproducibility.

4. Chlorine and nitrogen are both detected in neutral electrolyte solution. Neutrality (pH 7) is maintained by the mixed bed resin supplied by Tracor for removing ions from the circulating electrolyte. Detection of chlorine is optimum in slightly acidic solution, achieved by using a 1:4 mixture of anion (Duolite APA-366) and mixed bed (Duolite ARM-381) resins (FDA PAM, Section 315.42 (2)). Ions exchange resin should be extracted in a Soxhlet apparatus with water and the alcohol to be used with it prior to use.

5. A 50% (v/v) mixture of n-propanol in deionized water is recommended as the conductivity solvent. Methanol, isopropanol, and ethanol have also been used as the alcohol. Reducing the percentage of alcohol increases the sensitivity, especially in the nitrogen mode. Cell wall wetting may also be impaired, however, and flow irregularities may result in increased noise and poorer reproducibility. Twenty to thirty percent alcohol in water is usually the lowest practical choice for the best signal to noise ratio in the nitrogen mode. Methanol (100%) has been successfully used in the chlorine mode.

6. The quartz combustion tube is ¼ in. (0.6 cm) o.d. (4 mm or 2 mm i.d.) x approximately 7½ in. (19 cm) long. Separate tubes should be conditioned and maintained for nitrogen and chloride modes, respectively. Do not interchange the tubes once they have been used. A tube used in the chloride mode should be first heated to a dull red over its entire surface with a propane/oxygen torch to burn off surface impurities. This pretreatment, which has to be done only once, shortens the time required to reach maximum response and reduces peak tailing. In the nitrogen mode, the tube should not be treated as above.

7. The nickel catalyst is approximately 100 in. (254 cm) of 8 mil wire wrapped in a bundle 1-3/8 (3.5 cm) in. long. The catalyst should be inserted in the tube after the tube is installed and with reaction gas (H_2) turned on. The catalyst must be centered in the hot zone of the furnace. Normally, some response for 10 ng of atrazine should be obtained in just a few minutes after initial setup of standard operation conditions. However, the time required to reach maximum sensitivity may vary from one hour to

overnight depending on the condition of the catalyst. If the system is not used for long periods of time, it is advisable to have only reaction gas flowing over the catalyst. If the catalyst should slip so that a portion of it is outside the hot zone (expecially downstream), some of the converted sample could be adsorbed by contact with the metal. For this reason, it is wise to spread the ends of the wire bundle so it fits snugly in the furnace tube and to avoid bumping the instrument. Conversion of nitrogen to ammonia is increased by greater nickel surface area, but the amount of nickel wire is limited by the size of the combustion tube.

8. When changing modes of operation, also change or clean the 1/16 in. (0.16 cm) o.d. Teflon tubing between the combustion tube and the cell to prevent salt formation and sample loss in the line. In addition, install the proper ion exchange tube.

9. The Tracor Standard Sample contains atrazine (10 ng/μl) and aldrin (5 ng/μl) in methanol. In the nitrogen mode with a strontium hydroxide scrubber, only one peak for atrazine is observed (Fig. 1, Section 4,C,(2)). In the chloride mode, a peak representing the single chlorine in atrazine and a larger peak for aldrin should be obtained (Fig. 2, Section 4,C,(2).

10. The strontium hydroxide scrubber must be used in the nitrogen mode to eliminate any interference from acid gases. Insert a plug of the scrubber approximately ½ in. (1.3 cm) long, such that the outside edge of the plug is ¼ in. (0.6 cm) inside the furnace. To maintain stability, routinely replace the scrubber after 50-100 analyses.

11. Choice of solvent for sample and reference materials can be critical with the HECD, because several common solvents have adverse effects on the detector. Hydrocarbon solvents are considered preferable. When operated in the nitrogen mode, the detector responds to acetonitrile, even at low levels and even though the majority of the solvent is vented. The injection should be vented for at least two minutes if there is acetonitrile in the solution. Methylene chloride as the injection solvent depletes the strontium hydroxide scrubber needed for nitrogen mode operation and makes the detector inoperative. A vent time of 3 minutes is needed when using methylene chloride. Ethyl acetate often contains trace amounts of acetic acid, which also depletes the scrubber if this solvent is injected without venting. A minimum vent time of 2 minutes is recommended. Chlorine mode operation precludes the use of halogenated solvents in the HECD. See also Subsection II above

for a description of the problems encountered when using solvents containing oxygen.

VII. MAINTENANCE AND TROUBLESHOOTING:

Consult the Tracor Operation and Service Manual, the FDA PAM Section 315.6, and the EPA Pesticide AQC Manual, Section 5,G,b. Some further operating characteristics and maintenance instructions by Bayer are described in Section 5,G,b of the EPA Pesticide AQC Manual. The greater time and attention required for maintenance of the HECD compared to the EC and FPD is a disadvantage of the detector.

Problems encountered in the use of the HECD can usually be recognized prior to significant deterioration in performance and can often be solved simply and immediately if the analyst is familiar with the chemistry of the detector. Common problems in nitrogen detection include poor linearity and peak shape. Poor linearity is usually caused by neutralization of the NH_4CH. This is due to insufficient basicity of the conductivity solvent and/or exhausted scrubber. Neutralization problems are readily recognized by a sharp dip in the baseline just prior to the peak, followed by a negative dip after the peak that gradually increases to the baseline (Fig. 5). The peak may be totally negative if the solvent is acidic or the quantity of nitrogen compound is very small. Peak tailing is usually due to a contaminated scrubber, contaminated transfer line from the furnace to the cell, deactivated catalyst, or the presence of acidic reaction products that are not removed by the scrubber. The presence of acidic reaction products is also normally indicated by negative peaks. Negative peaks are prevented by using a properly packed ion exchange tube, high purity gases, and an efficient scrubber. Other solutions are obvious and include replacing the catalyst, ion exchange resin, scrubber, and transfer line as required. A "dirty" combustion tube can cause peak tailing and loss of sensitivity.

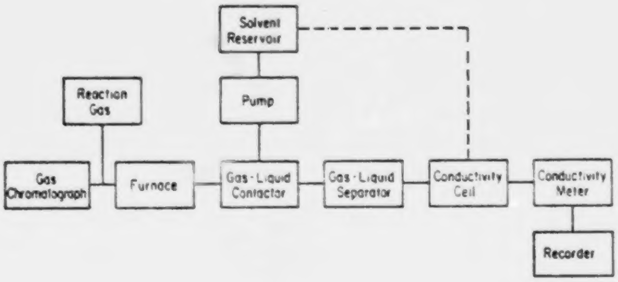

Solvent Reservoir
Pump
Reaction Gas
Gas Chromatograph
Furnace
Gas-Liquid Contactor
Gas-Liquid Separator
Conductivity Cell
Conductivity Meter
Recorder

Fig. 1. Block diagram of the electrolytic conductivity detector.

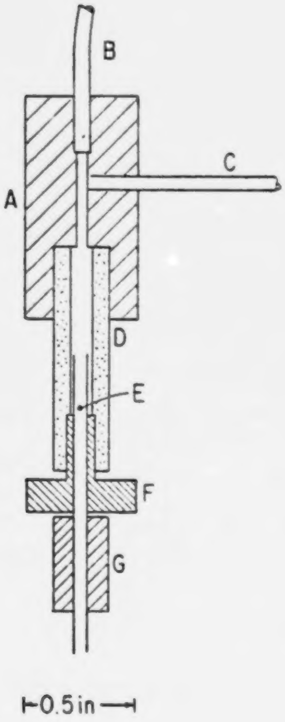
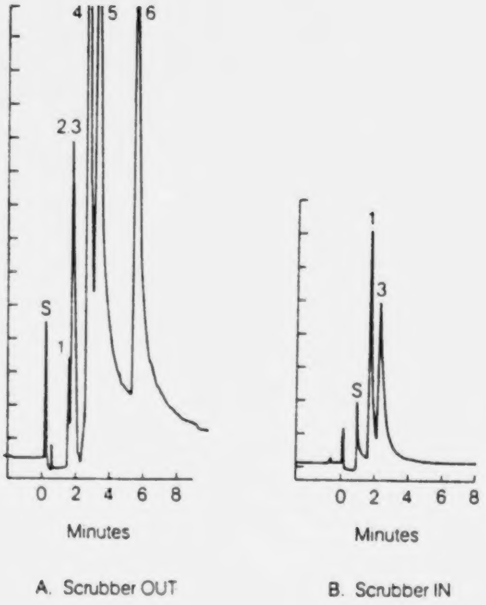

B
C
A
D
E
F
G
0.5 in

Fig. 2. Microelectrolytic conductivity detector cell assembly. A, gas-liquid contactor; B, Teflon solvent delivery tube; C, Teflon reaction products delivery tube; D, stainless steel detector block; E, solvent vent; F, Teflon insulator sleeve; G, gas-liquid exit tube and center electrode.

Sample Peak	Identity
S	Solvent
1	Atrazine (10 ng)
2	Lindane (100 ng)
3	Caffeine (10 ng)
4	Heptachlor (100 ng)
5	Aldrin (100 ng)
6	Dieldrin (100 ng)

Conditions

Column: 6 x ¼" Glass packed with 3% OV-1 on Chromasorb WHP 80/100 mesh
Col. Temp: 200° C
Carrier: H_2 at 50 cc/min
Chromatograph: Model 550
Detector: Hall Model 700
Mode: Nitrogen
Catalyst: Nickel wire
Furnace Temp: 850° C
Sensitivity: 10 x 4



A. Scrubber OUT

B. Scrubber IN

Fig. 3. Hall detector selectivity.

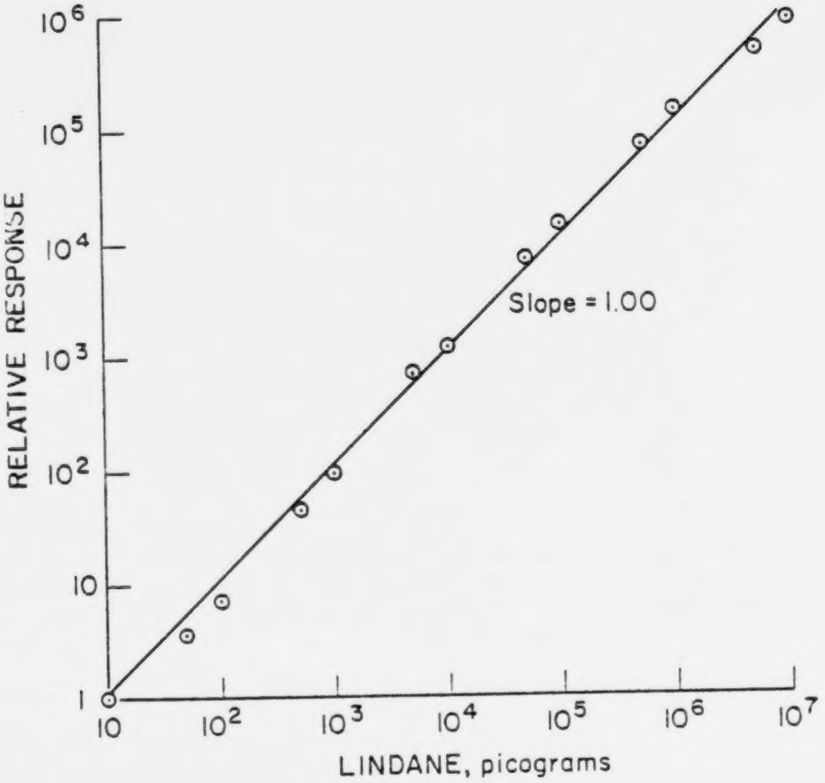


Fig. 4. HECD linearity-halogen mode.

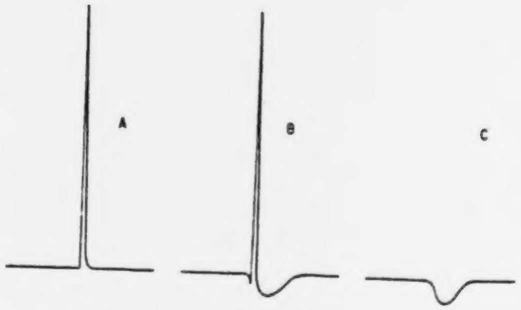


Fig. 5. Peak shapes obtained for nitrogen-containing compounds: A, normal peak; B, peak obtained with an insufficiently basic conductivity solvent; C, peak obtained with an acidic conductivity solvent.

GAS CHROMATOGRAPHY - HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

SAMPLE QUANTITATION AND INTERPRETATION

Methods of quantitation with the HECD are similar to those used with the electron capture (Section 4,A,(4)) and flame photometric (Section 4,B,(4)) detectors.

As with other element selective detectors such as the FPD (Section 4,B,(4), V), interpretation is greatly simplified with the HECD compared to the electron capture detector. Figures 1 and 2 compare this selectivity to the N-P detector (Section 4D) and the sulfur mode of the FPD.

Figure 1 shows that the HECD does not respond to levels of hydrocarbons (e.g., 100 µg of octadecane) that elicit significant response with the N-P detector. The HECD is specific for nitrogen; the response depends solely on the nitrogen content of the molecule detected. It is even possible to tune the HECD to only certain types of nitrogen compounds.

Figure 2 compares the response of the HECD in the sulfur mode to that of the FPD. The detectors display similar selectivities for the quantities of material shown, but the selectivity of the FPD would be reduced by a factor of three if the response were linearized. Linearization of the output of the FPD also enhances peak tailing. The output of the HECD is linear and suffers none of these disadvantages.

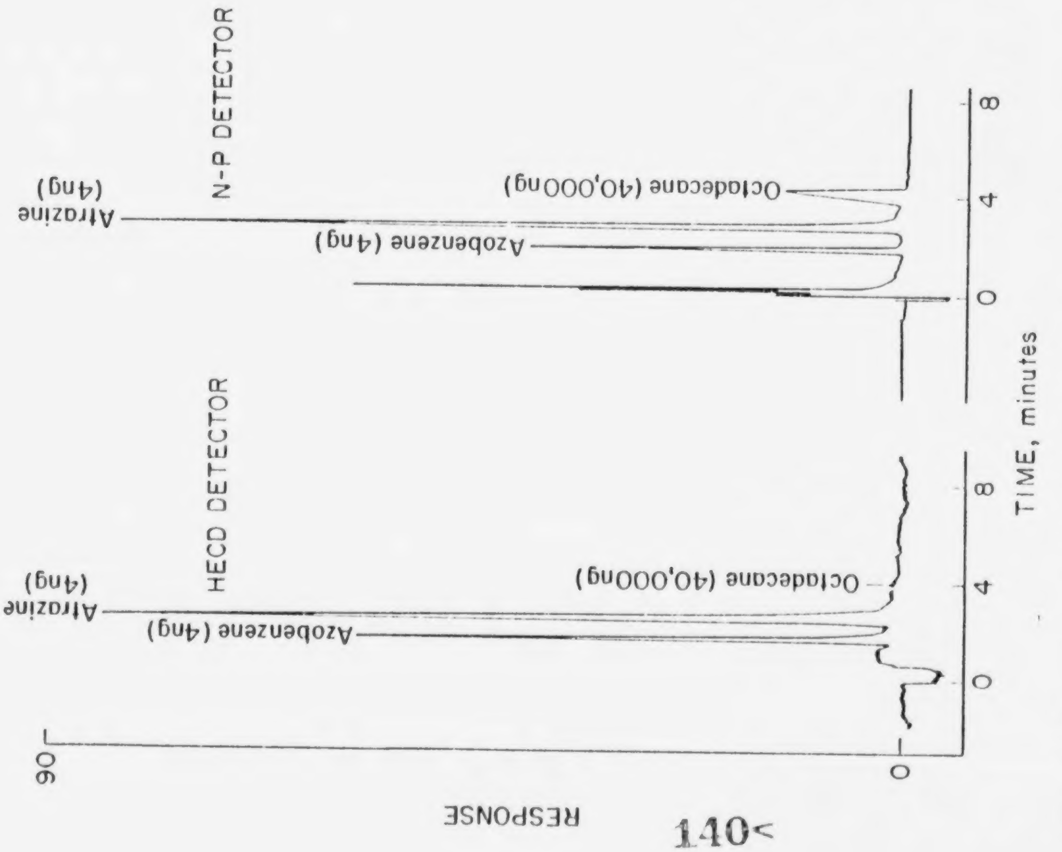


Fig. 1. Comparison of selectivity of 700A HECD and the N-P detector in their nitrogen modes.

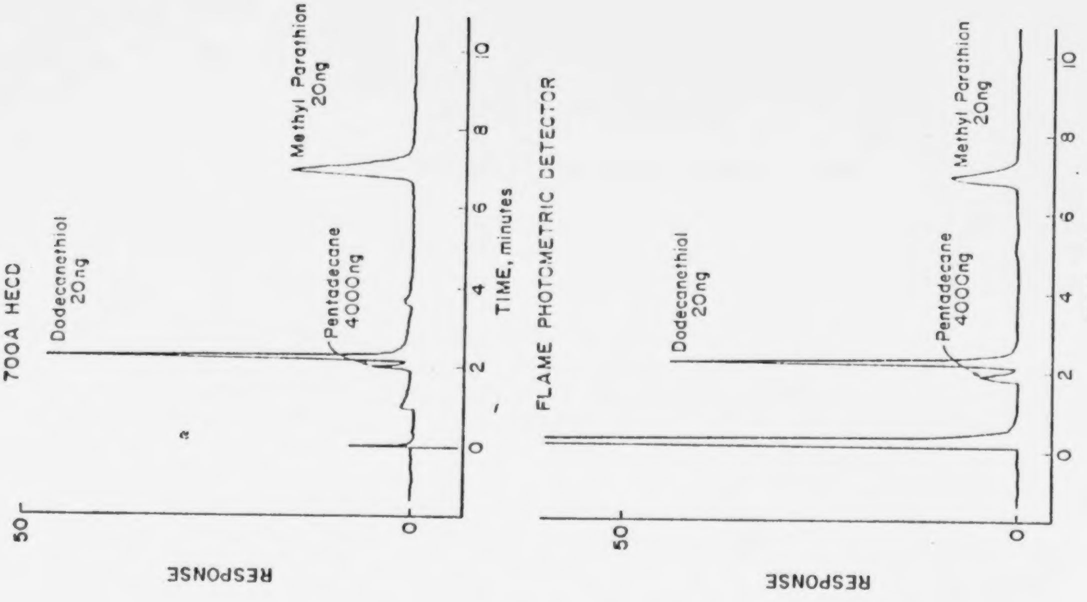


Fig. 2. Comparison of selectivity of 700A and FPD in their sulfur modes.

RETENTION DATA AND CHROMATOGRAMS OF CARBAMATE PESTICIDES ON CARBOWAX 20M-MODIFIED SUPPORTS WITH DETECTION BY THE HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

REFERENCE

Hall, R. C., and Harris, D. E., J. Chromatogr. 169, 245 (1979).

CONDITIONS:

Instrument	Tracor Model 560 gas chromatograph
Detector	Tracor Model 700 HECD
Electrolyte	15% isopropanol in water, 0.5 ml/minute
Transfer line temperature	200°C
Furnace temperature	720°C
Hydrogen reaction gas flow	80 ml/minute
Columns	Glass, 6 ft. (1.8 m) x 2 mm i.d., silanized with Supelco Sylon-CT, and ends plugged with silanized glass wool
Support	Commercial bonded Carbowax 20M, designated Ultra-Bond (RFR, Hope, RI)
Liquid phase coating	By evaporation technique, using rotary evaporator operated at 20 rpm for solvent removal
Column packing	By use of slight vacuum and gentle tapping with plastic rod
Column conditioning	At 190°C for 24 hours with normal carrier gas flow
Helium carrier gas flow	25 ml/minute

Retention Indices for Carbamate Pesticides

Relative to Carbofuran on Ultra-Bond and Coated Ultra-Bond

Column temperature is 170°

Compound*	Purity***	Ultra Bond	3% OV-101	1% OV-17	1% Carbowax 20M	1% OV-210	0.5% OV-210 + 0.65% OV-17
EPTC	99.5	--	0.20	0.08	0.07	--	--
Butylate	99.5	--	0.25	0.09	0.07	--	--
Pebulate	99.0	--	0.25	0.12	0.09	--	--
Vernolate	99.0	--	0.28	0.12	0.08	--	--
Propham	100.0	0.19***	0.31	0.19	0.22	--	0.22
Diallate	99.0	0.20***	0.67	0.31	0.21	0.32	0.28
Meobal	99.0	0.33	0.59	0.42	0.52	0.56	0.50
CDEC	99.5	0.34	0.66	0.40	0.30	0.40	0.37
Pyramat	98.0	0.35	0.62	0.43	0.29	0.39	0.36
Trillate	99.5	0.53	1.01	0.48	0.26	0.39	0.38
Propoxur	98/99	0.55	0.55	0.48	0.53	0.63	0.52
2,3,5-Landrin	98.0	0.60	0.69	0.51	0.58	0.65	0.58
Chlororopham	99.5	0.61	0.66	0.45	0.59	0.56	0.55
Bux	98.0	0.78	1.04	0.72	0.71	0.75	0.71
Terbutol	98.0	0.82	1.47	0.91	0.66	0.82	0.78
3,4,5-Landrin	98.0	0.85	0.94	0.78	0.85	0.88	0.85
Benthiocarb	98.0	0.85	1.80	1.26	0.32	0.75	1.02
Aminocarb	98.0	0.93	1.07	0.89	0.95	1.02	0.94
Mexacarbate	99.0	0.98	1.32	0.98	0.94	1.02	0.96
Carbofuran	99.5	1.00	1.00	1.00	1.00	1.00	1.00
SWEP	98.0	1.36	1.19	0.97	1.47	1.19	1.23
Dimetilan	98.0	1.37	1.79	1.93	1.38	1.86	1.64
Methiocarb	99.0	2.10	2.25	2.13	2.28	1.96	2.20
Carbaryl	99.5	2.75	2.48	2.41	3.10	2.81	2.82

*Compounds are listed by common names.

**Standards came from the EPA Protection Agency, Health Effects Research Laboratory, Environmental Toxicology Division, Research Triangle Park, NC 27711, USA

***Column temperature is 150°C.

Retention Parameters for the More Highly Volatile Carbamate Pesticides on Ultra-Bond

t_R = retention time; t'_R = retention relative to butylate

Compound	Purity	Temperature (°C)					
		90°		100°		120°	
		t_R(min)	t'_R	t_R(min)	t'_R	t_R(min)	t'_R
EPTC	99.5	3.27	0.69	2.00	0.73	1.09	0.87
Butylate	99.5	4.72	1.00	2.75	1.00	1.25	1.00
Vernolate	99.0	5.29	1.12	3.06	1.11	1.42	1.14
Pebulate	99.0	6.00	1.27	3.44	1.25	1.55	1.24

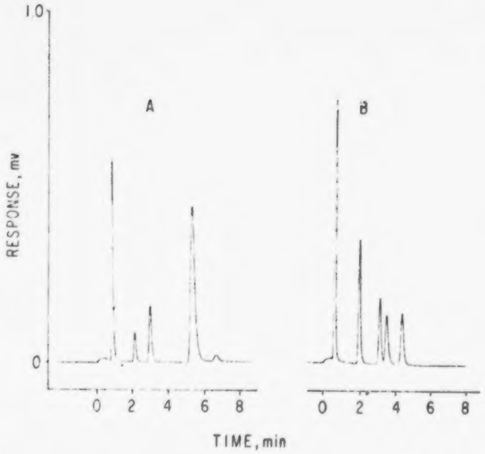


Fig. 1. Chromatograms of carbamate pesticides separated on a 3% OV-101 on Ultra-Bond column operated at 170°. Sensitivity: 10 × 8. Compounds in order of elution are: (A) butylate, CDEC carbofuran, Dimetilan and methiocarb; (B) EPTC, chlorpropham, triallate, SWEP and terbutol. Sample quantity: 10 ng each.

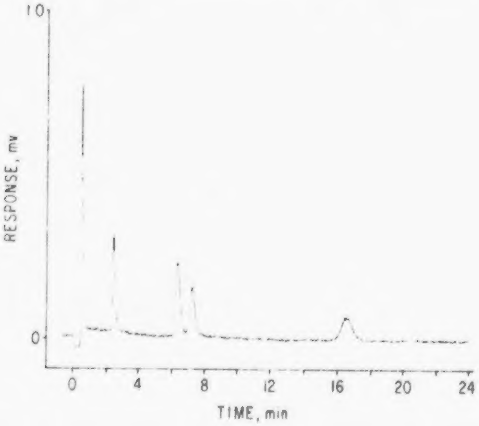


Fig. 2. Chromatogram of carbamate pesticides separated on a 1% Carbowax 20M column operated at 170°. Sensitivity 10 × 8. Compounds in order of elution are EPTC, chlorpropham, triallate, SWEP and terbutol. Sample quantity: 10 ng each.

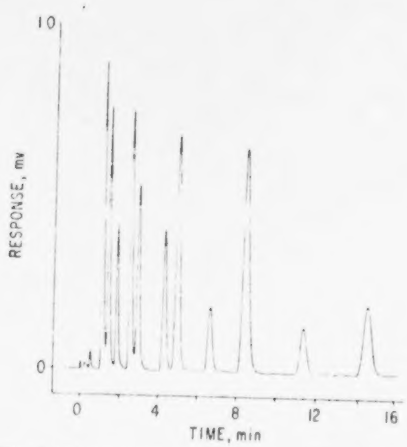


Fig. 3. Chromatogram of carbamate pesticides separated on a 0.65% OV-17 + 0.5% OV-210 on Ultra-Bond column operated at 170°. Sensitivity: 10 × 8. Compounds in order of elution are: Propham, diallate, triallate, meobal, 3,4,5-Landrin, carbofuran, mexacarbate, SWEP, Dimetilan, methiocarb and carbaryl. Sample quantity: 10 ng each with the exception of diallate at 5 ng.

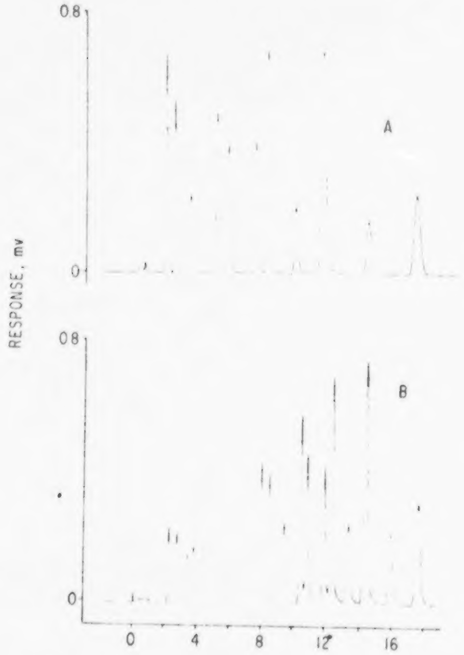


Fig. 4. Chromatogram of carbamate pesticides separated on a 0.65% OV-17 + 0.5% OV-210 on Ultra-Bond column. (A) Temperature-programmed from 115–175° at 10°/min. Sensitivity: 10 × 8. Compounds in order of elution: propham, diallate, triallate, meobal, 3,4,5-Landrin, carbofuran, mexacarbate, SWEP, Dimetilan, methiocarb and carbaryl. Sample quantity: 10 ng except for diallate at 5 ng. (B) Temperature-programmed from 110–185° at 10°/min. Sensitivity: 10 × 8. Compounds in order of elution are: EPTC, butylate, vernolate, pebulate, propham, diallate, triallate, meobal, 3,4,5-Landrin, carbofuran, mexacarbate, SWEP, Dimetilan, methiocarb and carbaryl. Sample quantity same as in A.

SUMMARY OF RESULTS:

A wide variety of carbamate pesticides can be chromatographed as the intact compounds, using Carbowax 20M-modified supports with or without additional liquid phase coatings. It is important that moderate column temperatures (<185°C) and relatively short analysis times be used. Collection of chemical-ionization mass spectra (isobutane reaction gas) of the parent carbamates of all compounds injected proved that among the 24 pesticides that could be readily chromatographed, carbaryl was the only compound that exhibited significant degradation (≈ 50%). In all other cases, the results of the CI-MS study indicated that the compounds detected by the electrolytic conductivity detector were the intact carbamates. The retention time of carbaryl was considerably longer than that of the other pesticides, which probably contributed to its degradation. Eleven carbamate pesticides can be separated on an OV-210/OV-17 mixed phase column under isothermal conditions. A total of 15 carbamates can be separated with baseline resolution on the same column with temperature programming.

GAS CHROMATOGRAPHY - NITROGEN - PHOSPHORUS (N-P) DETECTOR

I. INTRODUCTION:

Alkali flame ionization detectors (AFID) for selective analysis of phosphorus- and nitrogen-containing pesticides have been described and discussed in Section 313 of the FDA PAM and Section 5,E of the EPA Pesticide AQC Manual. The flame in the AFID serves the dual purposes of volatilizing the alkali salt source and ionizing the sample. Because the amount of hydrogen determines the flame temperature and, therefore, the extent of the above processes, small changes in hydrogen flow rate affect the detector response considerably. The flame temperature is also influenced by the flow rates of carrier gas and air. In actual practice, it is difficult to maintain gas flows within required tolerances for stable operation at high detector sensitivities.

The difficulty of maintaining very accurate gas (especially hydrogen) flow rates and the problems associated with the continually changing salt surface have resulted in decreased interest by pesticide analysts in the AFID in favor of "flameless" alkali sensitized detectors. These detectors, which have been recently sold under the name "nitrogen-phosphorus (or N-P) detector" because of their selective response to these elements, offer an order of magnitude improvement in sensitivity and selectivity. However, the low cost and ease of conversion of common flame ionization detectors to AFID operation makes this detector still attractive for analyses not requiring the higher stability of the flameless detector.

For general information on the gas chromatograph, GC columns, and sample quantitation and interpretation, consult Section 4,A.

REFERENCES:

1. Rapid Procedure for Preparation of Support Bonded Carbowax 20M Gas Chromatographic Column Packing, Moseman, R. F., J. Chromatogr., 166, 397-402 (1978).

2. Ionization Detector for GC with Switchable Selectivity for Carbon, Nitrogen, and Phosphorus, Kolb, B., Auer, M., and Pospisil, P., J. Chromatogr., 134, 65 (1977).

3. Analytical Performance of a Novel Nitrogen-Sensitive Detector and its Applications with Glass Open Tubular Columns, Hartigan, M. J., Purcell, J. E., Novotny, M., McConnell, M. L., and Lee, M. L., J. Chromatogr., 99, 339 (1974).

4. Study of the Nitrogen Response Mode of the Thermionic Rubidium Silicate Detector, Lubkowitz, J. A., Glajch, J. L., Semonian, B. P., and Rogers, L. B., J. Chromatogr., 133, 37 (1977).

II. DESCRIPTION OF N-P DETECTORS:

Figure 1 shows a schematic diagram of the Perkin-Elmer N-P detector used by R. F. Moseman of the EPA (reference 1 above) with support bonded Carbowax 20M columns (Section 4,A,(7)).

This detector and those available from Varian, Hewlett-Packard, and Tracor have similarities in the position of the alkali source above the detector jet, use of cylindrical collector electrodes, application of a negative potential to the source, and use of air and hydrogen only in quantities necessary to produce a low temperature plasma (rather than a flame) surrounding the source. The four commercial detectors are, however, distinct in geometry, alkali source, and electrical technique used for heating the source. The Perkin-Elmer detector uses a rubidium glass (silicate) bead source, the Hewlett-Packard uses an unspecified alkali salt contained in a ceramic reservoir, the Varian uses a proprietary alkali-ceramic bead, and the Tracor uses a mixture of alkali salts in a silica gel matrix. The sources have a relatively short bead life of about three months.

The N-P detector is similar in basic design to the AFID and can be considered as a modified AFID with an electrically heated source that is operated with reduced hydrogen and air flows. The plasma generated around the electrically heated beam is responsible for ionizing the sample. Initial installation and positioning of the bead is critical for optimum response. The bead temperature is easily set by a control knob. Thus, the system becomes more stable and has a wider linear range. Figure 1 shows a schematic diagram of the Perkin Elmer nitrogen-phosphorus detector. When this detector is used in the "phosphorus only" mode, the bead heating circuit is switched off, and the flame is ignited.

III. MECHANISM OF SELECTIVITY:

As in the case of the AFID, the mechanism of response of the N-P detector is not fully understood. However, a brief probable explanation of the mechanism of selective detection of the most widely studied Perkin-Elmer detector follows:

N-P Mode - (Sensitivity to nitrogen and phosphorus.) The N-P mode uses a negatively polarized jet and low hydrogen flow rate. Because of the lack of a hot flame, organic compounds do not burn completely. Rather, a partial pyrolysis takes place, producing intermediate stable CN radicals from nitrogen-containing organic compounds. The radicals take on an electron from the alkali, resulting in a symmetrical cyanide ion and a positive alkali ion. The alkali ion is recaptured by the bead, while the cyanide ion migrates to the collector electrode and liberates an electron. Collection of the electrons creates the specific response. A similar mechanism has been formulated for phosphorus, except that PO and/or PO_2 are assumed to be the intermediate radicals. It should be emphasized that there is no mode of the detector selective for nitrogen only; there is strong response to phosphorus plus nitrogen in the N-P mode.

P-Mode - (Sensitive to phosphorus only.) A hot flame exists because of an increased hydrogen flow rate, and the jet of the detector is gounded. The organic compounds are fully burned, and the electrons produced by the normal combustion process are conducted to ground. The combustion products of phosphorus react with the alkali on the surface of the bead and produce ions that are captured by the collector electrode, thus producing the response. Nitrogen compounds give a reduced response in this mode.

IV. RESPONSE CHARACTERISTICS:

Compared with the flame ionization detector, the N-P detector is about 50 times more sensitive for nitrogen and 500 times more sensitive for phosphorus. The sensitivity for three pesticides is illustrated in Figure 2. All of the compounds contain phosphorus, and diazinon also contains nitrogen. The sensitivity of detection calculated from the chromatogram for malathion is 6×10^{-14} g/second, or, calculated for P, 6×10^{-15} g/second. Nanogram to picogram quantities of most nitrogen compounds can be routinely determined, and selectivity is high if no phosphorus-containing compounds are co-injected. Sensitivity of the detector depends on detector background. The background is affected by bead heating current, gas flow rates, and condition of the bead. Operation of the detector at a fixed background current requires only occasional adjustment of the detector and has resulted in very uniform response with time. The hydrogen, air, and carrier (nitrogen or helium) flow rates should be optimized for maximum signal to noise ratio for the pesticide(s) of interest.

Figure 3 shows the wide linear range for the pesticide malathion. Selectivity of the N-P detector relative to organic molecules containing N or P atoms depends somewhat on the analytical conditions and the type of molecule. However, it has always been found to be better than 1:5000. This is important not only for positive identification of residues but also to eliminate the interference of the large solvent peak that might overlap early peaks in trace analysis when a nonselective detector is used. Figure 1 in Section 4,C,(4) illustrates the selectivity of the N-P detector.

V. GLC COLUMNS:

The N-P detector is not usable with columns of liquid phases containing halogen, phosphorus, or nitrogen (OV-210, XE-60, stabilized DEGS). Columns that have been used successfully include support bonded Carbowax 20M (Section 4,A,(7)), 3% OV-1, 8% Apiezon L, 5% Carbowax 20M, and 3% OV-17.

Figure 4 shows the separation and detection of pg levels of three triazine herbicides. Figure 5 compares detector response to nitrogen and hydrocarbon. The chromatogram demonstrates a nitrogen:carbon selectivity of 3 x 10^5.

VI. INSTALLATION, OPERATION, MAINTENANCE:

Consult the operations manual of the particular detector to be used. An advantage of the N-P detector is a relatively low degree of required maintenance.

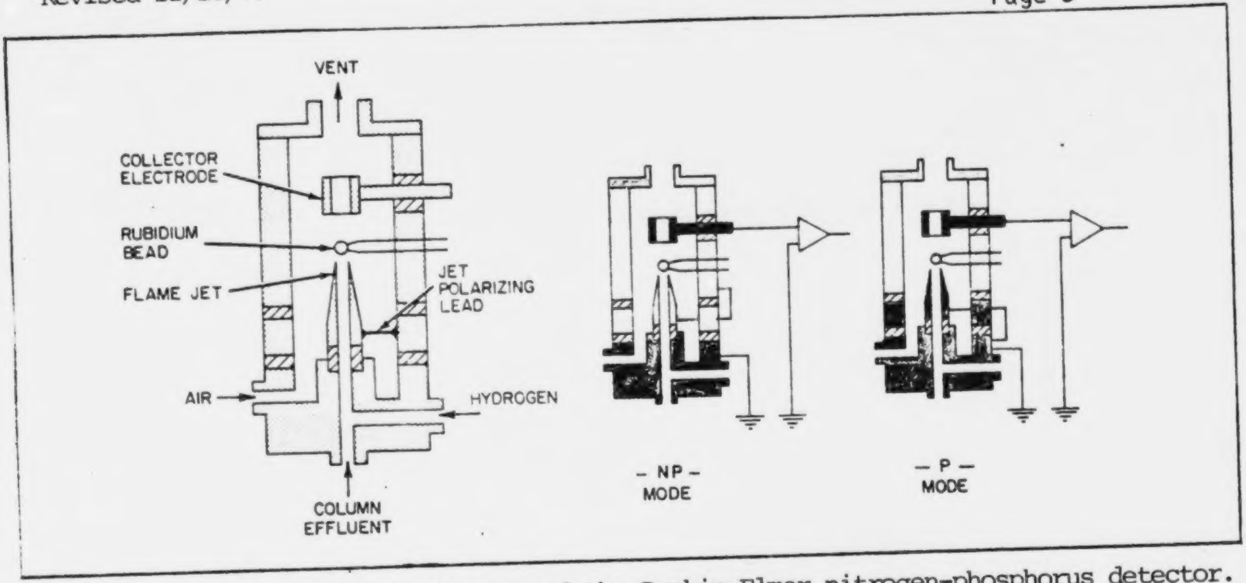

VENT
COLLECTOR ELECTRODE
RUBIDIUM BEAD
FLAME JET
JET POLARIZING LEAD
AIR
HYDROGEN
COLUMN EFFLUENT
– NP – MODE
– P – MODE

Fig. 1. Left: schematic diagram of the Perkin-Elmer nitrogen-phosphorus detector. Right: the two possible modes of operation. Parts with negative polarity are indicated in a light shading, and parts with positive polarity in black. Hatched area represents insulation. The electrical heating of the rubidium glass bead is not indicated.

Column: 6 ft. x 0.08 in. ID glass, containing 3% SE-30 on Chromosorb W HP 80/100 mesh.

Column temperature: 210 °C.

Sample volume: 1 μl.

DIAZINON (10.3% P; 9.2% N)	1×10^{-10}g
MALATHION (9.4% P)	1×10^{-10}g
ETHION (16.1% P)	1.3×10^{-10}g

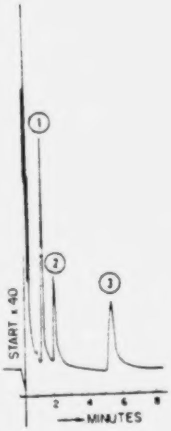


Fig. 2. Chromatogram of pesticides.

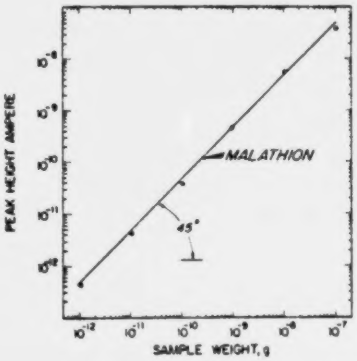


Fig. 3. Linearity plot of the nitrogen-phosphorus detector for malathion.

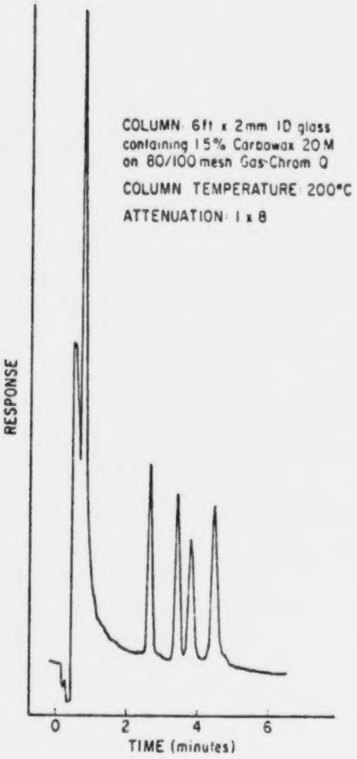


Fig. 4. Chromatogram of 200 pg each of atrazine, simazine, propazine, and prometryne.

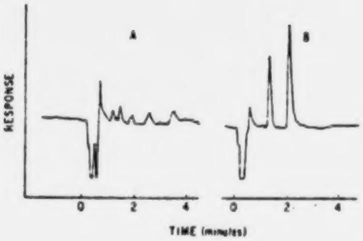


Fig. 5. Selectivity of the N-P detector. A: 5 ng each of C_{17}, C_{18}, C_{19}, and C_{20} normal hydrocarbons; B: 200 pg of atrazine and 100 pg of methyl parathion. Attenuation, 10 x 4.

☆ MODIFICATION OF MILLS, ONLEY, GAITHER METHOD FOR THE DETERMINATION OF MULTIPLE ORGANOCHLORINE PESTICIDES AND METABOLITES IN HUMAN OR ANIMAL ADIPOSE TISSUE

I. INTRODUCTION:

This procedure combines some of the extraction features of the de Faubert Maunder et al method and the Florisil partitioning and cleanup basics of the Mills et al procedure. The modified procedure has been collaboratively studied over a period of years and has been found to yield interlaboratory relative standard deviation values of 15 percent or better for the chlorinated pesticidal compounds most commonly found in the fat of humans and animals.

REFERENCES:

1. de Faubert Maunder, M. J., Egan, H., Godly, E. W., Hammond, E. W., Roburn J., and Thompson, J. The Analyst, 89: 168, 1964.
2. Mills, P. A., Onley, J. H., and Gaither, R. A., J.A.O.A.C. 46, 186-191, 1963.

II. PRINCIPLE:

A 5 g. sample is dry macerated with sand and Na_2SO_4 and the fat is isolated by repetitive extractions with pet. ether. Pesticide residues are extracted from the fat with acetonitrile and then partitioned back into pet. ether by aqueous dilution of the acetonitrile extract. Pet. ether extract is concentrated to 5 ml by Kuderna-Danish evaporation and transferred to a Florisil column for successive elutions with 6% and 15% ethyl ether/pet. ether. The respective eluates are both concentrated to suitable volumes in K-D evaporators and the final extracts are examined by electron capture gas-liquid chromatography.

III. EQUIPMENT:

1. Gas chromatograph equipped for electron capture detection. Specific GLC columns and recommended operating parameters are given in Section 4,A.

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*This method, with appropriate modifications, may be used for the analysis of other tissues if original sample size is adequate.

2. Aluminum foil, household type.

3. Beakers, 250 ml, stainless steel or heavy duty glass.

4. Beakers, 250 ml, Griffin low form.

5. Stirring rods, glass 10 mm diameter.

6. Water bath with temperature adjustment of 90-100°C.

7. Filter paper - Whatman No. 1, 15 cm diameter.

8. Funnels, glass, ca 60 ml diameter.

9. Separatory funnels - 125 ml and 1 liter, Kimble 29048-F, or equiv.

10. Chromatographic columns - 25 mm o.d. x 300 mm long, with Teflon stopcocks, without fritted glass plates, Kontes 420530, Size 241.

11. Filter tubes, 150 x 24 mm, such as Corning #9480.

12. Erlenmeyer flasks - 500 ml capacity.

13. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, such component bearing the following stock numbers:

    a. Flask, 500 ml, stock #K-570001

    b. Snyder Column, 3-ball, stock #K-503000

    c. Steel springs, 1/2 in., stock #K-662750

    d. Concentrator tubes, 10 ml, size 1025, stock #K-570050

14. Modified micro-Snyder columns, 19/22, Kontes K-569251.

15. Glass beads, 3 mm plain, Fisher #11-312 or equivalent.

16. Glass wool - Corning #3950 or equivalent.

IV. REAGENTS:

1. Petroleum ether - Pesticide Quality, redistilled in glass, b.p. 30° - 60°C. (See Note 7, p. 10)

2. Diethyl ether - AR grade, peroxide free, Mallinckrodt #0850 or the equivalent. The ether must contain 2% (v/v) absolute ethanol. Some of the AR grade ethers contain 2% ethanol, added as a

stabilizer, and it is therefore unnecessary to add ethanol unless peroxides are found and removed.

NOTE: To determine the absence of peroxides in the ether, add 1 ml of freshly prepared 10% KI solution to 10 ml of ether in a clean 25-ml cylinder previously rinsed with the ether. Shake and let stand 1 minute. A yellow color in the ether layer indicates the presence of peroxides which must be removed before using. See Misc. Note 4 at end of procedure. The peroxide test should be repeated at weekly intervals on any single bottle or can as it is possible for peroxides to form from repeated opening of the container.

3. Eluting mixture, 6% (6+94) - purified diethyl ether 60 ml is diluted to 1000 ml with redistilled petroleum either and anhydrous sodium sulfate (10-25 g) is added to remove moisture.

4. Eluting mixture, 15% (15+85) - purified diethyl ether 150 ml is diluted to 1000 ml with redistilled petroleum ether and dried as described above.

NOTE: Neither of the eluting mixtures should be held longer 24 hours after mixing.

5. Florisil, 60/100 mesh, PR grade, to be stored at 130°C until used.

NOTES: 1. In a high humidity room, the column may pick up enough moisture during packing to influence the elution pattern. To ensure uniformity of the Florisil fractionation, it is recommended to those laboratories with sufficiently large drying ovens that the columns be packed ahead of time and held (at least overnight) at 130°C until used.

2. Florisil furnished to the contract laboratories by the RTP, NC laboratory on order, has been activated by the manufacturer, and elution pattern data is included with each shipment. However, each laboratory should determine their own pesticide recovery and elution pattern on each new lot received, as environmental conditions in the various laboratories may differ somewhat from that in RTP, NC. Each new batch should be tested by the procedure described in Section 3,D for assurance that the operator can obtain recoveries and compound elution patterns comparable to the data given on the accompanying table.

6. Acetonitrile, reagent grade, saturated with pet. ether.

   NOTE: Occasional lots of CH_3CN are impure and require redistillation. Generally, vapors from impure acetonitrile will turn litmus paper blue when the moistened paper is held over the mouth of the bottle.

7. Anhydrous sodium sulfate, reagent grade granular, Mallinkrodt stock #8024 or the equivalent.

   NOTE: When each new bottle is opened, it should be tested for contaminants that will produce peaks by Electron Capture Gas Liquid Chromatography. This may be done by transferring ca 10 grams to a 125 ml Erlenmeyer flask, adding 50 ml pet. ether, stoppering and shaking vigorously for 1 minute. Decant extract into a 100 ml beaker and evaporate down to ca 5 ml. Inject 5 µl into the Gas Liquid Chromatograph and observe chromatogram for contaminants. When impurities are found, it is necessary to remove them by extraction. This may be done by using hexane in a continuously cycling Soxhlet extraction apparatus or by several successive rinses with hexane in a beaker. The material is then dried in an oven and kept in a glass-stoppered container.

8. Sodium Chloride solution, 2%, from reagent grade NaCl.

   NOTE: See Note for sodium sulfate, Step 7, above.

9. Sand, quartz, which has been acid washed and extracted with hexane to produce a zero background in the determinative step.

10. MgO-Celite mixture (1:1) weigh equal parts of reagent grade MgO and Celite 545 and mix thoroughly.

11. Hexane, redistilled, pesticide quality.

V. SAMPLING:

The majority of human adipose tissue samples are taken during autopsy and the chemist has little or no control over the sampling process. Wherever possible, it should be recommended to the autopsy physician that the sample be placed in a glass container with Teflon or foil-lined screw cap. Plastic bags should be avoided as traces of impurities such as phthalates may contaminate the sample and result in many spurious chromatographic peaks when the final sample is examined by electron capture GLC.

VI. SAMPLE PREPARATION & EXTRACTION:

1. On a cupped sheet of lightweight aluminum foil, weigh 5 grams of the previously minced fat. Transfer entire cup to a 250 ml stainless steel or heavy duty glass beaker.

2. Add ca 10 grams of clean, sharp sand, ca 10 grams of anhydrous Na_2SO_4 and 1.0 ml of hexane solution containing 200 nanograms of aldrin.

   NOTE: The aldrin is added here for the dual purpose of (1) providing a built-in retention marker for direct peak identification on all chromatograms of the first fraction extract, and (2) as a quantitative recovery check for the procedure. This inoculation should, of course, not be made if aldrin is suspected to be in the substrate.

3. Grind the mixture with a heavy glass rod and continue adding portions of Na_2SO_4 to give a uniform, dry granular mass.

4. Add 50 ml of pet. ether and warm carefully on a water bath with continuous stirring until solvent boils gently.

   NOTE: Some laboratories have reported satisfactory recoveries resulting from the use of hexane instead of pet. ether as the extracting solvent. In all probability, hexane would function as a satisfactory substitute but the modification has not been subjected to collaborative study, and therefore no supporting data is available to validate this hypothesis.

5. Place Whatman No. 1 filter paper in glass funnel and rinse several times with pet. ether. Place funnel over previously tared 250-ml beaker and transfer extract to funnel by decantation.

6. Extract the contents of the first beaker with two more 50-ml portions of pet. ether as described in Steps 4 and 5.

7. Transfer insoluble material to the filter paper and ringe beaker and paper with a final 10 ml of pet. ether.

8. Place beaker on a 40°C water bath and evaporate just to dryness under a stream of nitrogen. Check odor to be sure all solvent is removed and allow to cool to room temperature in a dessicator.

9. Weigh beaker and record for calculation of percent fat in the sample.

10. Accurately weigh between 2.8 and 3.0 grams of the fat obtained in Step 9 into a 125-ml separator. Add 12 ml of pet. ether previously saturated with acetonitrile.

    NOTE: In the case of highly saturated animal fat, it will be necessary to add 17 ml of pet. ether to the separator. In such a case the amount of acetonitrile used in the partitioning step should be increased to 40 ml.

VII. LIQUID-LIQUID PARTITIONING:

1. Add 30 ml of acetonitrile, previously saturated with pet. ether. Stopper funnel and shake vigorously for 2 minutes.

2. Allow phases to separate and draw off the acetonitrile layer into a 1-liter separator containing 700 ml of a 2% solution of NaCl and 100 ml of pet. ether.

3. Similarly extract the pet. ether layer in the 125-ml separator three more times with 30-ml portions of acetonitrile, combining all acetonitrile extracts in the 1-liter separator.

4. Stopper, invert 1-liter separator, vent off pressure and mix by shaking for two minutes, releasing pressure as required.

5. Allow the layers to separate and drain aqueous layer into a second 1-liter separator.

6. Add 100 ml pet. ether to second separator, and after a 30-second vigorous shaking, discard aqueous phase and transfer pet. ether phase into first 1-liter separator.

7. Wash pet. ether with two 100-ml portions 2% NaCl and discard the aqueous washings.

8. Prepare a 2-inch column of anhydrous, granular Na_2SO_4 in a 150 x 24 mm filter tube and position over a 500-ml K-D evaporator fitted with a 10-ml grad. concentrator tube containing one glass bead. Dry the pet. ether by filtering through this column. Rinse the separator twice with 10-ml portions of pet. ether and finally rinse down sides of the filter tube with 10 ml pet. ether.

9. Attach a 3-ball Snyder column to the top of the K-D evaporator and place in a 90-100°C water bath. Approximately 1-1/2 inches of the concentrator tube should be below the surface of the water.

10. Concentrate the extract to ca 5 ml, rinse down the sides of K-D evaporator and the ground glass joint with a total of 3 ml pet. ether. Reconcentrate extract to ca 5 ml under a gentle stream of nitrogen at room temperature.

VIII. FLORISIL FRACTIONATION:

1. Prepare a chromatographic column containing 4 inches (after settling) of activated Florisil topped with 1/2 inch of anhydrous, granular Na_2SO_4. A small wad of glass wool, preextracted with pet. ether, is placed at the bottom of the column to retain the Florisil.

   NOTES: 1. If the oven is of sufficient size, the columns may be prepacked and stored in the oven, withdrawing columns a few minutes before use.

   2. The amount of Florisil needed for proper elution should be determined for each lot of Florisil.

2. Place a 500-ml Erlenmeyer flask under the column and prewet the packing with pet. ether (40-50 ml, or a sufficient volume to completely cover the Na_2SO_4 layer).

   NOTE: From this point and through the elution process, the solvent level should never be allowed to go below the top of the Na_2SO_4 layer. If air is introduced, channeling may occur, making for an inefficient column.

3. Using a 5-ml Mohr or a long disposable pipet, immediately transfer the tissue extract (ca 5 ml) from the evaporator tube onto the column and permit it to percolate through.

4. Rinse tube with two successive 5-ml portions of pet. ether, carefully transferring each portion to the column with the pipet.

   NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down the sides of the column.

5. Prepare two Kuderna-Danish evaporative assemblies complete with 10 ml graduated evaporative concentrator tubes. Place one glass bead in each concentrator tube.

6. Replace the 500-ml Erlenmeyer flask under each column with a 500-ml Kuderna-Danish assembly and commence elution with 200 ml of 6% diethyl ether in pet. ether (Fraction I). The elution rate should be 5 ml per minute. When the last of the eluting solvent reaches the top of the Na_2SO_4 layer, place a second 500-ml

Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in pet. ether (Fraction II).

7. To the second fraction only, add 1.0 ml of hexane containing 200 nanograms of aldrin, place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract until ca 5 ml remain in the tube.

8. Remove assemblies from bath and cool to ambient temperature.

9. Disconnect collection tube from Kuderna-Danish flask and carefully rinse joint with a little hexane.

10. Attach modified micro-Snyder column to collect tubes, place tubes back in water bath and concentrate extracts to 1 ml. If preferred, this may be done at room temperature under a stream of nitrogen.

11. Remove from bath, and cool to ambient temperature. Disconnect tubes and rinse joints with a little hexane.

    NOTE: The extent of dilution or concentration of the extract at this point is dependent on the pesticide concentration in the substrate being analyzed and the sensitivity and linear range of the Electron Capture Detector being used in the analysis (See Section 4,A).

12. Should it prove necessary to conduct further cleanup on the 15% fraction, transfer 10 grams MgO-Celite mixture to a chromatographic column using vacuum to pack. Prewash with ca 40 ml pet. ether, discard prewash and place a Kuderna-Danish receiver under column. Transfer concentrated Florisil eluate to column using small portions of pet. ether. Force sample and washings into the MgO-Celite mixture by slight air pressure and elute column with 100 ml pet. ether. Concentrate to a suitable volume and proceed with Gas Liquid Chromatography.

    NOTE: Standard Recoveries should be made through column to ensure quantitative recoveries.

IX. ASSESSMENT OF EXTRACT CONCENTRATION:

1. Inject 5 μl of each fraction into the gas chromatograph for the purpose of determining the final dilution. If all peaks are on-scale and quantifiable, it will not be necessary to proceed with any further adjustment in concentration. With human fat, however, it is probable that there will be several sizable on-scale peaks and one or more off-scale peaks in the 6% fraction.

2. If off-scale peaks are obtained in either fraction it will be necessary to dilute volumetrically with hexane to obtain a concentration that will permit quantitation of those peaks from a 5 μl injection.

   NOTE: A 5-ml dilution of a 3.0 gram sample containing .01 ppm of a given pesticide will yield 30 picograms of the pesticide per 5-microliter injection. Provided the detector is operating at average sensitivity, it should be possible to obtain quantifiable peaks of most compounds likely to be present at this concentration.

X. MISCELLANEOUS NOTES:

1. The two fractions from the Florisil column should never be combined for examination by Gas Liquid Chromatography. By so doing, a valuable identification tool is voided.

2. Meticulous cleaning of glassware is absolutely essential for success with this procedure. All reagents and solvents must be pretested to ensure that they are free of contamination by electron capturing materials at the highest extract concentration levels. Reagent blanks should be run with each set of samples.

3. The method, as described, is known to be capable of producing recoveries of most of the chlorinated pesticides of from 85 to 100%. Each laboratory should conduct its own recovery studies to make certain of its capability to achieve this recovery range. A clue may be obtained from the recovery of the aldrin spike. The recovery of this compound should not be less than 70%.

4. For the removal of peroxides from the ethyl ether, place an appropriate volume in a separatory funnel and wash it twice with portions of water equal to about 1/2 the volume of ether. The washed ether is shaken with 50 to 100 ml of saturated NaCl solution and all of the aqueous layer is discarded. The ether is then transferred to a $ flask containing a large excess of anhydrous sodium sulfate and shaken vigorously on a mechanical shaker for 15 minutes. This treatment should not be attempted on ether containing ethanol, as the amount of ethanol that would remain is indeterminate.

5. If the presence of malathion is suspected, it is necessary to pass 200 ml of 50% diethyl ether in pet. ether through the Florisil column into a third K-D evaporator assembly, concentrating the eluate as described for the 6% and 15% eluates.

6. Table 1 gives the elution pattern for a number of common pesticides. On occasion it may be observed that a portion of a given compound may elute into a different fraction than the one given. For example, some operators have difficulty eluting all the dieldrin in the 15% fraction. This is generally caused by either moisture in the system or the use of solvents of different polarity than those specified in the reagent list. For example, it is essential that the diethyl ether contain 2% (v/v) ethanol. Ether without the ethanol or with too much would expectedly result in an altered elution pattern.

7. If this method is used for the detection and quantitation of organophosphorous compounds, some special factors must be considered. The presence of any peroxides in the ethyl ether and/or impurities in the pet. ether can result in extremely low recoveries. Recovery efficiency should be predetermined on standard mixtures containing the specific compounds of interest. If low recoveries are obtained, it may be necessary to try an alternate manufacturer's pet. ether.

8. If the presence of HCB is suspected in the sample, the analyst would be well advised to apply the confirmatory procedure described in Section %,A,(11),(b) since recoveries by the method described in this section (5,A,(1),(a), are expectedly poor. If HCB is detected in a significant number of routine samples, a modification in the extraction stage (Subsection VI,10) would prepare for the confirmation contingency and save some time. In Step 10 weigh 3.4 grams of fat and transfer to a 13 ml grad., conical centrifuge tube. Add pet. ether to bring the volume to the 10 ml mark. Stopper securely and mix on a rotary mixer 30 minutes at ca 50 rpm. Quantitatively transfer 2 ml of the extract to a small vial, seal and set aside under refrigeration for possible use in confirmation. Transfer the remaining 8 ml of extract to a 125 ml separator, rinsing tube with two 2 ml portions of hexane. Proceed with Subsection VII.

TABLE 1. A COMPILATION OF FLORISIL ELUTION PATTERNS AND RECOVERY DATA OF PESTICIDES

INTRODUCTION:

The data contained in the following table were copies from "Analytical Behavior Data for Chemicals Determined Using AOAC Multiresidue Methodology for Pesticide Residues in Foods," McMahon, B., and Burke, J. A., J. Assoc. Off. Anal. Chem., 61, 640 (1978). Reproduction here is intended to provide the reader with the elution characteristics and recovery potential of many pesticides and industrial chemicals in addition to those that are normally found in adipose tissue.

The elution behavior and recovery data for many of these compounds were obtained from fatty foods (FDA PAM, Sections 211.1/231.1(6); Official Methods of Analysis of the AOAC, 12th ed., (1975), Sections 29.001, 29.002, 29.005, 29.008-29.010, 29.012, 29.014, 29.015, 29.018; and Changes in Methods, J. Assoc. Off. Anal. Chem., 59, 471 (1976), Sections 29.B01-29.B06), but because of the similarity of the extraction and Florisil partitioning steps used in analyzing adipose tissue, it would be expected that results would be very similar or identical in the analysis of human or animal fat.

Circumstances under which the data were obtained varied widely. Different data have been validated by many analysts or by only one, with or without sample present, through complete methods or through individual procedures of a method. Much of the data has been proven valid during a number of years of routine use of the methodology in many laboratories.

When complete data on the behavior of a compound are unavailable, the available data are given and the missing information is indicated.

Available information is presented on elution of compounds from the Florisil column with additional eluants in cases where the 6% and 15% ethyl ether-petroleum ether eluants were insufficient.

CODE:

C: Complete (>80%) recovery; may apply to the complete method or to only the Florisil column elution by the specific eluant(s) shown.

P. Partial (>80%) recovery; may apply to the complete method or to only the Florisil column elution by the specific eluant(s) shown. Approximate percent recovery expected is given in parentheses, when known.

V. Variable recoveries or inconsistent elution patterns.

NR. Not recovered; may apply to the complete method or to only the Florisil column elution by the specific eluant(s) shown.

ND. No data; indicates compound has not been tested through complete procedure.

FLORISIL ELUTION NOTATIONS:

1. Percentages in this column refer to percent ethyl ether in petroleum ether eluants in 200 ml portions in which the compounds eluted. Unless otherwise indicated, percentages above 15% were used in addition to the usual 6% and 15% eluants.
2. Appearance of C, P, or NR plus the appropriate eluant(s) indicates that the information was obtained during testing of Florisil elution only.
3. Appearance of appropriate eluant alone indicates that the information was obtained during testing of the entire method.

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Acarol	C	C, 15 + 50%
Acetyl tributyl citrate	P	50%
Acetyl triethyl citrate	P	50%
Acetyl tris (2-ethyl hexyl)citrate	ND	50%
Alachlor (Lasso)	ND	NR, 6, 15%
Aldrin	C	6%
Allidochlor (Randox)	ND	NR, 6, 15%
Anilazine (Dyrene)	P	15%
Aramite	NR	P, 15%
Aroclor 1016	C	6%
Aroclor 1221	C	6%
Aroclor 1242	C	6%
Aroclor 1248	C	6%
Aroclor 1254	C	6%
Aroclor 1260	C	6%
Aroclor 1262	C	6%
Aroclor 4465	C	6%
Aspon	ND	6%
Atrazine	ND	C, 50%
Azinphos-ethyl (Ethyl Guthion)	ND	P, 50%
Azinphos-metnyl (Guthion)	ND	NR, 6, 15%
Benfluralin (benefin)	C	C, 6%
Bensulide (Prefar)	C	C, 50%
Benzoylprop-ethyl (Suffix)	ND	NR, 6, 15, 50%
α-BHC	C	6%
β-BHC	C	6%
γ-BHC (lindane)	C	6%
δ-BHC	C	6, 15% V
Binapacryl	P(65)	P(75-90), 15%
Bis(2-ethoxyethyl)phthalate	ND	ND
Bis(2-methoxyethyl)phthalate	ND	ND
Bis(trichloromethyl)disulfide	ND	6%
Bis(3,3,5-trimethylcyclohexyl) phthalate	ND	15%
Bomyl	ND	ND
Bromacil	ND	NR, 6, 15, 50%
Bromophos	ND	6%
Bromophos-ethyl	ND	6%
Bulan	P(75)	15%
Butoxy ethyl ester 2,4-D	P	15%
Butoxy ethyl ester 2,4,5-T	P	15%
Butyl benzyl phthalate	P(70)	C, 15 + 50%
n-Butyl ester 2,4-D	P(10)	15%
n-Butyl ester 2,4,5-T	P	15%
Butyl isodecyl phthalate	C	15 + 50%
Butyl octyl phthalate	ND	15 + 50%
Butyl phthalyl butyl glyocate	P	50%
Captafol (Difolatan)	ND	P(64), 50%
Captan	ND	50% V
Captan epoxide	ND	NR, 6, 15%

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Carbophenothion (Trithion)	P(60)	6% V
Carbophenothion oxygen analog	ND	ND
CDEC (Vegadex)	C	6%
Cereclor S-45 (chlorinated paraffin)	C	6%
Cereclor S-52 (chlorinated paraffin)	C	6%
Chlorbenside	C	6%
Chlordane (technical)	C	6%
Chlordane (cis)	C	6%
Chlordane (trans)	C	6%
Chlordecone (Kepone)	P	P, 15, 50% V
Chlorfenvinphos	ND	ND
α-Chlorfenvinphos	NR	ND
Chlornidine (Torpedo)	P(70)	C, 15%
Chlorobenzilate	P(75)	C, 15 + 50%
Chloroneb	ND	6%
Chloropropylate	C	C, 15 + 50%
Chlorowax 40 (chlorinated paraffin)	C	6 + 15%
Chlorowax 500C (chlorinated paraffin)	C	6%
Chlorowax 70 (chlorinated paraffin)	C	6%
Chlorothalonil (Daconil 2787)	NR	NR, 6, 15, 50%
Chlorpham (CIPS)	C	15%
Chlorpyrifos (Dursban)	C(74-83)	6%
Chlorpyrifos (Dursban) oxygen analog	ND	ND
Chlorthion	C	15%
Clorafin 40 (chlorinated paraffin)	P	6 + 15%
Clorafin 50 (chlorinated paraffin)	C	6 - 15%
Coumaphos (Co-Ral)	ND	NR, 6, 15, 30%
CP-40 (chlorinated paraffin)	C	6 + 15%
Cresyl diphenyl phosphate	C	50%
Crotoxyphos (Ciodrin)	ND	ND
Crufomate (Ruelene)	ND	ND
Cumylphenyl diphenyl phosphate	C	50%
Cyanazine (Bladex)	ND	ND
Cypromid	ND	NR, 6, 15%
Dacthal	P	15%
o,p'-DDE	C	6%
p,p'-DDE	C	6%
o,p'-DDT	C	6%
p,p'-DDT	C	6%
DEF	P(60)	C, 15 + 50%
Demeton (Systox)	ND	ND
Diablo 700X (chlorinated paraffin)	C	6%
Dialifor	P(50)	C, 15%

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Diallyl phthalate	ND	15 + 50%
Diazinon	C	15%
Dibutoxyethyl phthalate	C	15 + 50%
Di-*n*-butyl phthalate	C	C, 15 + 50%
Dicapthon	C	C, 15 + 50% V
Dichlobenil (Casoron)	C(80)	C, 15%
Dichlofenthion (Nemacide)	P(>70)	C, 6%
Dichlone	NR	NR, 6, 15, 50%
p-Dichlorobenzene	C	6%
m-Dichlorobenzene	C	6%
o,*p*'-Dichlorobenzophenone	C	15%
p,*p*'-Dichlorobenzophenone	C	15%
Dichlorvos (DDVP)	NR	NR, 6, 15, 50%
Dicloran (Botran)	P(50)	P, 15%, C, 15 + 20%
Dicofol (Kelthane)	P	6, 15% V
Dicrotophos (Bidrin)	ND	ND
Dicyclohexyl phthalate	C	15 + 50%
Di-*n*-decyl phthalate	ND	15 + 50%
Dieldrin	C	15%
Diethyl phthalate	P	15 + 50%
Di-2-ethylhexyl isophthalate	C	15%
Di-2-ethylhexyl phthalate	C	C, 15 + 50%
Diisobutyl phthalate	ND	C(ca 80), 15 + 50%
Diisodecyl phthalate	C	15 + 50%
Diisohexyl phthalate	ND	C, 15 + 50%
Diisononyl phthalate	ND	15%
Diisooctyl phthalate	C	C, 15 + 50%
Diisopropyl phthalate	ND	15 + 50%
Dilan	P(65)	15%
Dimethoate	ND	ND
Dimethoate oxygen analog	ND	ND
Dimethyl isophthalate	ND	NR
Dimethyl phthalate	ND	P, 6, 15, 50%
Di(methylisobutylcarbinyl) phthalate	ND	15%
Di(1-methylheptyl)adipate	C	6%
Dinocap (Karathane)	P(60)	P(75), 15%
Dinonyl phthalate	ND	15 + 50%
Di-*n*-octyl phthalate	C	C, 15 + 50%
Dioxathion (Delnav)	ND	ND
Dipentyl phthalate	ND	15%
Diphenamid	ND	ND
Diphenyl phthalate	ND	15 + 50%
Dipropyl phthalate	ND	15 + 50%
Disulfoton (Di-Syston)	ND	P(25-40), 6%
Disulfoton oxygen analog (demeton-S)	ND	ND
Disulfoton sulfone	ND	ND

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Diuron	ND	NR, 6, 15, 50%
Endosulfan I (Thiodan I)	C	15%
Endosulfan II (Thiodan II)	C	15 + 50%
Endosulfan sulfate	C	50%
Endrin	C	15%
Endrin alcohol	C	C, 15 + 50%
Endrin aldehyde	C	C, 15 + 50%
Endrin ketone (Delta Keto 153)	C	25% (following 6% only)
EPN	C	15%
Epoxyhexachloronorbornene	ND	6%
EPTC (Eptam)	ND	P, 15%
Ethion	C	6%
Ethoprop (MOCAP)	P(45)	50%
2-Ethylhexyl diphenyl phosphate	C	50%
Ethyl hexyl ester 2,4-D	C	15%
Ethyl phthalyl ethyl glycolate	NR	NR, 6, 15, 50%
Famphur	ND	ND
Fenitrothion (Sumithion)	C	15%
Fensulfothion (Dasanit)	ND	ND
Fensulfothion oxygen analog	ND	ND
Fensulfothion sulfone	ND	ND
Fenthion	ND	6, 15%
Folpet (Phaltan)	P(50)	C, 15 + 50% V
Fonofos (Dyfonate)	C	6%
Genite 923	C	C, 15%
Halowax 1001 (chlorinated naphthalene)	C	6%
Halowax 1013	C	6%
Halowax 1014	C	C, 6%
Halowax 1031	C	6%
Halowax 1051	C	6, 15%
Halowax 1099	C	C, 6%
Halowax 2141	C	6%
Hatcol 149 (mixed alkyl phthalates)	ND	15 + 50%
Hatcol 190	ND	15 + 50%
Heptachlor	C	6%
Heptachlor epoxide	C	6%
Heptachloronorbornene	C	ND
Hexachlorobenzene	P(60)	6%
Hexachloro-1,3-butadiene	P	6%
Hexachlorocyclopentadiene	ND	6%
Hexachloroethane	C	ND
Hexachloronorbornadiene	C	ND
Hexachlorophene	ND	NR, 6, 15, 50%
Hydroxy chloroneb	ND	ND
Isobenzan (Telodrin)	C	6%
Isobutyl ester 2,4-D	C	15%

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Isodecyl isooctyl phthalate	ND	15 + 50%
Isodrin	C	6%
Isooctyl ester 2,4-D	P(75)	15%
Isooctyl ester 2,4,5-T	C	15%
Isopropyl biphenyl	C	5%
Isopropyl ester 2,4-D	P(65)	15%
Isopropyl ester 2,4,5-T	C	15%
Korax (Lanstan)	ND	NR, 6, 15%
Leptophos (Phosvel)	C	C, 6%
Malathion	C	15, 50% V
Malathion oxygen analog	ND	ND
Merphos	C	6, 15, 50% V
Methidathion (Supracide)	P(50)	50%
p,p'-Methoxychlor	C	6%
o,p'-Methoxychlor	ND	6%
Methyl phthalyl ethyl glycolate	NR	NR, 6, 15, 50%
Methyl Trithion	C(depends on Florisil)	6% V
Mevinphos (Phosdrin)	ND	ND
Mirex	P(70)	6%
Mirex, 2,8-dihydro-(photoproduct)	ND	6%
Mirex, 10,10-dihydro-(photoproduct)	ND	6%
Mirex, 8-monohydro-(photoproduct)	ND	6%
Mirex, 10-monohydro-(photoproduct)	ND	6%
MO	C	6 + 15% V
Monobutyl phthalate	ND	15%
Monocrotophos (Azodrin)	ND	ND
Monuron	ND	NR, 6, 15, 50%
Naled	ND	ND
Neburon	ND	NR, 6, 15, 30%
Nitrofen (TOK)	C	C, 15%
Nonylphenyl diphenyl phosphate	C	50%
Octachlor epoxide (oxychlordane)	C	6%
Octachloro-dibenzo-p-dioxin	ND	NR, 6, 15%
Octachlorostyrene	ND	6%
Ovex (chlorfenson)	C	15%
Oxadiazon	P(75)	C, 15%
Parathion	C	15%
Parathion-methyl (methyl parathion)	C	15%
Parathion-methyl oxygen analog	ND	ND
Parathion oxygen analog	ND	ND
Paroil 1400V (chlorinated paraffin)	C	6%
Paroil 1500V	C	6%
Pentachloraniline	C	6%
Pentachlorobenzene	C	C, 6%
Pentachlorobenzoitrile	P(60)	15%
Pentachlorophenyl methyl sulfide	C	C, 6%

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
Perthane	C	6%
Perthane olefin	C	6%
Phenkapton	ND	6%
Phorate (Thimet)	P(80)	6%
Phorate oxygen analog sulfone	ND	ND
Phosalone	C	C, 50%
Phosmet (Imidan)	ND	ND
Phosphamidon	ND	ND
Phostex	ND	6%
Photodieldrin A	C	15%, final trace, 50%
Planavin	P(70)	P(50-80), 50%
Prolan	P(25)	15%
Prometryn	P(70)	P(67), 50%
Propachlor (Ramrod)	ND	NR, 6, 15%
Propanil (Stam F-34)	ND	NR, 6, 15%
Propazine	NR	C(80-94), 15 + 50% V
PX-316 (mixed alkyl phthalates)	ND	15 +50%
Quinotozene (PCNB)	C	6%
Ronnel (fenchlorphos)	C	6%
Ronnel oxygen analog	ND	ND
Schradan (OMPA)	ND	ND
SD 7438	C	C, 15%
Simazine	ND	C, 50%
Strobane	C	6%
Sulfotepp	P(65)	6 + 15 % V
Sulphenone	ND	50%
T-146 (mixed n-alcohol phthalates)	ND	15 + 50%
T-147	ND	15 + 50%
T-148	ND	15 + 50%
o,p'-TDE	C	6%
p,p'-TDE	C	6%
p,p'-TDE olefin	C	6%
Tecnazene (TCNB)	C	6%
Terbacil	ND	NR, 6, 15%
Terbufos (Counter)	ND	6%
Terbuthylazine	ND	15 + 50%
2,3,4,5-Tetrachloroanisidine	ND	6%
2,3,4,6-Tetrachloroanisidine	ND	6%
2,3,5,6-Tetrachloroanisidine	ND	6%
2,3,4,5-Tetrachloroanisole	ND	6%
2,3,4,6-Tetrachloroanisole	ND	6%
2,3,5,6-Tetrachloroanisole	ND	6%
1,2,3,4-Tetrachlorobenzene	C	ND
1,2,3,5-Tetrachlorobenzene	C	ND
2,3,4,5-Tetrachlorobenzene	C	ND
2,3,7,8-Tetrachlorodibenzo-p-dioxin	P(70)	p, 6, 15% V
2,3,4,5-Tetrachloronitroanisole	ND	6%

(continued)

TABLE 1. (continued)

Compound	Method Recovery	Florisil Elution
2,3,4,6-Tetrachloronitroanisole	ND	6%
2,3,5,6-Tetrachloronitroanisole	ND	6%
Tetrachlorvinphos (Gardona)	ND	ND
Tetradifon (Tedion)	C	15%
Tetraiodoethylene	P(65)	6%
Tetrasul	C	C, 6%
Thionazin (Zinophos)	NR	C(80), 15 + 50% V
Toxaphene (camphechlor)	C	6%
Tri(2-butoxyethyl)phosphate	NR	NR
Tributyl citrate	NR	ND
Trichlorobenzenes	P(60)	C, 6%
Tricresyl phosphate	C	50%
Triethyl citrate	NR	ND
Tri(2-ethylhexyl)phosphate	P	50%
Trifluralin	C	6%
Triphenyl phosphate	C	50%
Tris(1-bromo-3-chloroisopropyl) phosphate	P	50%
Tris(β-chloroethyl)phosphate	NR	NR
Tris(2,3-dibromopropyl)phosphate	NR	NR
Tris(1,3-dichloroisopropyl) phosphate	P	50%
Tris(dichloropropyl)phosphate	P(V)	50%
Tris(isopropylphenyl)phosphate	C	50%
Vernolate (Vernam)	ND	P, 15%
Zytron	C	6%

ANALYSIS OF ADIPOSE TISSUE
DETERMINATION OF HEXACHLOROBENZENE AND ☆ MIREX WITH CONFIRMATION OF HEXACHLOROBENZENE

I. INTRODUCTION:

The detection and quantitation of hexachlorobenzene (HCB) in adipose or other fatty tissues have posed problems to the analyst for two basic reasons: (1) in chromatography with electron capture detection, the retention characteristics of the HCB peak are quite similar to the alpha isomer of BHC (hexochlorocyclohexane) on a number of GC columns; (2) because of the unfavorable partition ratio of HCG (and mirex) in the acetonitrile/petroleum ether partition cleanup system, low recoveries are obtained using the multiresidue method outlined in Section 5,A,(1),(a). The procedure below offers improved recoveries and an HCB confirmatory derivative analysis. The confirmation scheme is especially useful for HCB because HCB elutes very early from the GC columns commonly used for pesticide residue analysis. Although the procedure was developed specifically for determination of HCB residues, it has proven useful for determination of mirex residues in adipose tissue.

REFERENCES:

1. Rapid Determination and Confirmation of Low Levels of Hexachlorobenzene in Adipose Tissue, Crist, H. L., Moseman, R. F., and Noneman, J. W., Bull. Environ. Contam. Toxicol. 14, 273-280 (1975).

2. Collaborative Study of an Improved Method for Hexachlorobenzene and Mirex and Hexachlorobenzene Confirmation in Adipose Tissue, Watts, R. R., Hodgson, D. W., Crist, H. L., and Moseman, R. F., J. Assoc. Off. Anal. Chem., submitted for publication. (Official First Action status has been granted by the AOAC.)

II. PRINCIPLE:

An adipose tissue sample is dissolved in hexane and applied directly to a Florisil column. The HCB and mirex residues are eluted with hexane and determined by direct EC-GC of the concentrated eluate. HCG residues are then confirmed by EC-GC analysis of a disubstituted ether derivative (bis-isopropoxytetrachlorobenzene; BITB) formed by reaction with 2-propanol. Mirex residues do not survive this reaction.

III. APPARATUS:

1. Gas chromatograph fitted with ^3H or ^{63}Ni electron capture detector and 1.8 m x 4 mm i.d. columns of 1.5% OV-17/1.95% OV-210 and 5% OV-210 on 80-100 mesh support. Operating parameters: column temperature, 200°C (OV-17/OV-210), 180°C (OV-210); detector (tritium) 210°C, (nickel) 300°C; inlet block 220°C; transfer line 220°C; carrier gas flow 60 ml/min.
2. Columns, glass, Chromaflex, size 241, 300 x 25 mm o.d., Kontes No. K-420530.
3. Kuderna-Danish concentrator assembly, Kontes No. K-570000, fitted with 25 ml graduated evaporative concentrator tube (K-570050), size 2525, 19/22).
4. Micro-Snyder column, Kontes No. K-569250.
5. Disposable pipets.
6. Compressed, gaseous nitrogen equipped with regulator valve for pressure reduction to approximately 5 lb/in^2.
7. Water bath, with temperature range of 50-100°C.
8. Glass wool, pre-extracted in a Soxhlet apparatus with hexane.
9. Vortex mini-mixer.

IV. REAGENTS AND SOLVENTS:

1. Hexachlorobenzene and mirex analytical reference standards, available from the Quality Assurance Section, U.S. EPA, ETD, HERL, MD-69, Reserach Triangle Park, NC 27711.
2. Pyridine, Burdick and Jackson, or equivalent, suitable for liquid or gas chromatography. Test the reagent for intereferences by using the derivatization procedure.
3. Potassium hydroxide, reagent grade; 10% solution in 2-propanol.
4. Sodium sulfate, anhydrous, granular. Soxhlet extract with hexane and oven dry at 130°C.
5. Sodium sulfate, 2% aqueous solution, prepared from pre-extracted reagent.
6. Florisil, PR grade, the Floridin Company, Berkeley Springs, WV. Prepare Florisil column by packing Chromaflex column with 100 mm

adsorbent and 12 mm Na SO on top [see Section 5,A,(1),(a), III, IV, and VIII]. Hold in 130°C ± 2°C oven for at least 16 hours prior to use. Remove stopcocks before placing columns in oven. Prewash with 50 ml hexane just before use.

7. Keeper solution, 1% paraffin oil in hexane.

8. Hexane, 2-propanol, pesticide quality, or equivalent.

V. PROCEDURE:

1. Accurately weigh 0.5 g of rendered or extracted fat in a 13 ml centrifuge tube.

2. Dissolve the fat in ca 0.5 ml of hexane and quantitatively transfer to a Florisil column prewashed with 50 ml of hexane. Rinse the sample tube with tow 0.5 ml portions of hexane and add each to the column.

3. Allow the column to drain until the solvent level is just at the top of the Na_2SO_4.

4. Rinse the column insides above the adsorbent bed with 2-3 ml of hexane.

5. Elute with 200 ml of hexane at a flow rate of 5 ml/minute.

   NOTE: The elution characteristics of each lot of Florisil should be tested for both compounds, and the elution volume should be adjusted if necessary.

6. Collect the eluent in the Kuderna-Danish assembly containing a 3 mm glass bead or carborundum chip in the 25 ml concentrator tube.

7. Immerse the concentrator tube in a boiling water or steam bath to about 1/3 of its depth and concentrate the extract to ca 10 ml.

8. Remove the K-D assembly from the bath, cool, and carefully remove the concentrator tube, rinsing the joint with ca 3 ml of hexane.

9. Place the tube under a nitrogen stream and reduce the extract volume to ca 3 ml. Do Not Allow to go to Dryness!!!

10. Rinse the sidewalls of the tube with hexane and adjust the volume to 5 ml. Stopper and Vortex mix one minute.

NOTES:

1. On the basis of the gas chromatographic analysis below, it may prove necessary to further dilute or concentrate the extract.

2. In addition to HCB and mirex, the 200 ml hexane fraction may contain heptachlor, aldrin, *p*,*p*'-DDE, *o*,*p*'-DDT, and PCBs. No attempt should be made to quantitate any compounds other than HCB that may appear in this eluate, as the elution may be incomplete and, therefore, give low recoveries.

VI. GAS CHROMATOGRAPHY:

Determine the amount of HCB and mirex in the sample by injecting 3-8 μl amounts of standards and samples into an OV-17/OV-210 GC column with the parameters stated in Subsection III,1. Alternatively, quantitate mirex on an OV-210 column. The RRT_A of HCB, the HCB derivative, and mirex are in the following table.:

RRT_A of Compounds (Conditions in Subsection III,1)

	5% OV-210	1.5% OV-17/1.95% OV-210
HCB	0.46	0.48
HCB derivative	N.D.[1]	0.86
Mirex	3.78	6.1

[1] Not determined.

Adjust sample volumes as required to produce major peak responses, so that no peak less than 20% f.s.d. is quantitated. Peak heights of standards and samples should not vary by more than 25%, and concentrations must fall within the linear range of the detector. It is best to work at the same attenuation setting for samples and standards.

VII. CONFIRMATION OF HCB BY DERIVATIZATION:

1. Add 5 drops of paraffin oil keeper solution to the sample (step 10, Subsection V) and place under a gentle nitrogen stream in a warm water bath. Continue evaporation until 0.1-0.2 ml of hexane remains.

   NOTE: At least three concentrations of the HCB standard should be derivatized along with samples. Choose concentrations that bracket the concentration of the HCB present in the sample as determined by the initial GC analysis. The responses of the HCB standard derivatives should be linear. Add 2-3 drops of paraffin oil keeper solution before evaporating to 0.1-0.2 ml.

2. Add 0.2 ml of pyridine and 0.5 ml of 10% KOH in 2-propanol, attach a modified micro-Snyder column to the concentrator tube, and place in a boiling water bath for exactly 45 minutes.

3. Remove the tube, cool under tap water, and add 10 ml of 2% Na_2SO_4 solution and exactly 2 ml of hexane. Stopper and mix vigorously for one minute.

   NOTE: The 2 ml of hexane should be delivered with a volumetric pipet, because this volume is used in calculating the amount of HCB residue.

4. After the phases have completely separated, inject 3-8 μl of the hexane extract (upper layer) into the gas chromatograph fitted and adjusted as described in Subsection III,1. An additional quantitative volume of hexane may be added, as estimated, to bring the BITB peak on scale. After such a dilution, the tube must be stoppered, again shaken, and time allowed for layer separation before sampling for GC. If the height of the BITB peak is less than 10% f.s.d., some further concentration is required by evaporation under a nitrogen stream. Quantitation is obtained by comparison of results to the reference standards of HCB carried through the derivatization procedure along with the unknown. Mirex is not recovered in the derivatization procedure.

   NOTE: Using the prescribed column temperature of 200°C, the RRT_A of the BITB peak on the OV-17/OV-210 GC column should be 0.86 (see the Table in Section VI).

VIII. RECOVERY RESULTS:

1. Studies by the authors of the original work (Reference 1) over a concentration range of 0.01 to 1.0 ppm have indicated recoveries from 86 to 107% when a 100 ml (rather than 200 ml) Florisil eluate is quantitated as specified in the earlier version of this method (see Table 1).

2. Tables 2-4 present a summary of the collaborative results (Watts et al., Reference 2) for the unknown standard solutions (8 ng/ml of HCB and 96 ng/ml of mirex), fat blank, spiked samples of HCB before and after derivatization, and spiked samples of mirex. Each value reported for fortified fat represents an average of the three repetitive determinations with the standard deviation of the three values directly underneath. Using these figures, the percent intralaboratory coefficient of variation (CV) for each spiking level and the average intralaboratory CV value are calculated and reported on the same horizontal line of the table. The interlaboratory results are given at the bottom of each column. Results marked with a 1 superscript have been determined to be "outlier" values as calculated by the Fitness Test method described in the EPA Quality Control Manual, Chapter 2, Section K,e.

The standard solution average result of 7.95 ng/ml for the direct HCB standard analysis represents a relative accuracy of 99.4%. The GCB interlaboratory average recoveries from Table 2 of 89.6, 87.4, and 92.6% (after blank value subtraction) for the 20.0, 33.3, and 50.0 ppb fortified samples represent good efficiencies over the range tested. The respective interlaboratory CV values ranged from an excellent 6.8% to a good 9.96%.

The results in Table 3 for the HCB confirmatory analysis by formation of the BITB derivative show three HCB interlaboratory average percent recoveries of 79.8, 78.8, and 76.9, respectively, for the 20.0, 33.3, and 50.0 ppb fortifications when corrected for the fat blank of 2.25 ppb. The respective interlaboratory CV values of 15.7, 18.6, and 19.0% demonstrate acceptable precision, although understandably not nearly as good as direct HCB determinations.

The Table 4 results for mirex indicate no difficulty with the unknown standard solution or the three fortification levels. The standard solution mean of 97.5 ng/ml represents an accuracy of 101.6% relative to the 96 ng/ml actual value. The three mirex average results, when corrected for the average blank of 27.5 ppb, yielded interlaboratory percent recovery values of 89.0, 90.2, and

92.3, respectively, for the 150, 300, and 500 ppb fortification levels. The respective interlaboratory CV values of 7.6, 16.5, and 18.1% represent excellent to acceptable precision values.

IX. MISCELLANEOUS NOTES:

1. Collaborator comments on the HCB derivative scheme indicated some unresolved problems with formation of only the disubstituted (BITB) derivative. Significant quantities of the monosubstituted derivative were often formed that prevented accurate quantitations. Collaborator No. 2 did not report derivative results for this reason, and large intralaboratory CV values in Table 3 also indicate the same problem. The derivative scheme is, therefore, recommended as qualitative and semi-quantitative confirmation of HCB.

2. The analyst may find that the hexane eluate will yield a clearly delineated peak of the precise RRT_A value for HCB. In this case, there may be little need for the derivatization step. However, for more reliable confirmation, the derivatization step is recommended. Early eluting compounds such as isomers of hexachlorocyclohexane (BHC) and heptachlor, which could present interfering peaks, are altered and do not interfere after derivatization. Aldrin, dieldrin, endrin, p,p'-DDE, and PCBs are not altered, but their normal elution characteristics pose no interference problems.

3. If the analyst has been alerted to the possible presence of HCB in the sample during a routine analysis by method 5,A,(1),(a), it is feasible to provide ahead of time for this contingency during the extraction step. See Miscellaneous Notes, Subsection 8, of Section 5,A,(1),(a).

TABLE 1. RECOVERY OF HCB FROM FORTIFIED CHICKEN FAT BY DIRECT ELUTION WITH 100 ML OF HEXANE[1]

Fat, mg	HCB Added, ng	HCB Recovered, ng	HCB Conc. µg/g	Recovery %
403	4	4.1	0.010	102
494	8	7.0	0.016	88
477	16	14.8	0.034	92
474	25	24.8	0.053	99
583	50	51.5	0.086	103
482	48	51.2	0.100	107
530	150	143	0.283	95
497	160	160	0.322	100
446	250	254	0.561	102
555	350	301	0.631	86
528	500	477	0.947	95
525	1000	1003	1.900	100

Mean 97.4%

Range 86-107%

Standard Deviation ±6.3%

[1] Crist et al. (Reference 1)

TABLE 2. HCB COLLABORATIVE RESULTS (WATTS ET AL., REFERENCE 2)

Fat Fortification (ppb)			20.0	33.3	50.0	20.0	33.3	50.0	
Lab	Unknown Std. (8 pg/µl) %	Fat Blank (ppb)	Average (ppb) Standard Deviation			Intralab Coefficient of Variation (%)			Average Intralab Coefficient of Variation (%)
1	8.0 100.0	2.80	22.0 1.00	31.3 3.06	50.3 7.77	4.5	9.8	15.4	9.9
2	7.44 93.0	2.95	21.2 0.29	35.9 8.72	51.9 10.22	1.4	24.3	19.7	15.1
3	7.75 96.9	2.05	20.7 2.01	32.3 2.17	49.6 1.42	9.7	6.7	2.9	6.4
4	12.5^1 156.3	2.00	22.7 5.85	32.3 4.04	50.0 22.72	25.8	12.5	45.4	27.9
5	8.0 100.0	2.20	19.60 0.40	31.0 2.65	51.5 3.32	2.0	8.5	6.4	5.6
6	7.5 93.8	1.45	18.6 2.11	30.3 1.15	38.9 0.81	11.3	3.8	2.1	5.7
7	7.28 91.0	5.58	17.3 4.62	29.7 1.03	42.4 2.31	26.7	3.5	5.4	11.9
8	7.7 96.3	2.70	21.7 2.08	31.7 2.52	46.3 3.21	9.6	7.9	6.9	8.1
9	9.3 116.3	7.0^1	28.8^1 10.83	40.7^1 5.14	55.5 13.18	37.6	12.6	23.7	24.6
10	7.9^1 98.9	3.02	17.8 4.48	27.9 1.82	34.8^1 5.20	25.2	6.5	14.9	15.5
11	9.0 112.5	3.70	22.1 2.46	34.0 3.14	47.7 0.55	11.1	9.2	1.2	7.2
12	7.6 95.0	0	21.6 1.15	32.8 1.51	53.8 1.12	5.3	4.6	2.1	4.0
Mean	7.95	2.58	20.5	31.7	48.9				
Mean (%)	99.4		89.6^2	87.4^2	92.6^2				
Std. Dev.	0.64		1.86	2.14	4.87				
Coef.Var.(%)	8.05		9.1	6.8	9.96				

[1] Outlier

[2] Mean Corrected for Blank

TABLE 3. HCB DERIVATIVE COLLABORATIVE RESULTS (WATTS ET AL., REFERENCE 2)

Fat Fortification (ppb)		20.0	33.3	50.0	20.0	33.3	50.0	
Lab	Fat Blank (ppb)	Average (ppb) Standard Deviation			Intralab Coefficient of Variation (%)			Average Intralab Coefficient of Variation (%)
1	3.0	19.3 1.15	26.3 5.51	37.0 9.54	6.0	21.0	25.8	17.6
2		--	--	--				
3	2.0	15.8 6.9	20.0 16.2	38.7 15.6	43.7	81.0	40.3	55.0
4	--	--	--	--				
5	4.0	21.3 2.31	34.0 6.24	53.0 5.2	10.8	18.4	9.8	13.0
6	--	17.7 2.2	26.6 2.5	35.7 1.9	12.4	9.4	5.3	9.0
7	7.54[1]	22.1 5.7	33.1 3.9	44.8 5.5	25.8	11.8	12.3	16.6
8	0	17.0 6.6	30.7 11.9	35.7 13.3	38.8	38.8	37.3	38.3
9	0	4.3[1] 3.9	7.0[1] 2.8	9.3[1] 5.2	90.7	40.0	55.9	62.2
10	2.86	14.7 2.0	23.3 4.4	31.3 6.2	13.6	18.9	19.8	17.4
11	3.9	21.2 2.5	36.1 1.7	52.8 1.1	11.8	4.7	2.1	6.2
12	--	14.9 1.2	26.7 1.3	37.7 4.8	8.1	4.9	12.7	8.6
Mean	2.25	18.2	28.5	40.7				
Mean (%)		79.8[2]	78.8[2]	76.9[2]				
Std. Dev.		2.86	5.30	7.74				
Coeff.Var.(%)		15.7	18.6	19.0				

[1] Outliers

[2] Mean Corrected for Blank

TABLE 4. MIREX COLLABORATIVE RESULTS (WATTS ET AL., REFERENCE 2)

Fat fortification (ppb)			150	300	500	150	300	500	
Lab	Unk. Std. (96 pg/µl) %	Fat Blank (ppb)	Average (ppb) Standard Deviation			Intralab Coefficient of Variation (%)			Average Intralab Coefficient of Variation (%)
1	89.8 93.5	10.0	148 5.29	287 9.54	490 54.8	3.6	3.3	11.2	6.0
2	96.2 100.2	32.3	166 5.10	317 61.0	523 66.0	3.1	19.2	12.6	11.6
3	92.0 95.8	15.7	177 14.8	341 21.6	568 20.0	8.4	6.3	3.5	6.1
4	103.0 107.3	20.0	160 23.8	212 30.2	349 131	14.9	14.2	37.5	22.2
5	96.0 100.0	8.0	156 1.53	290 16.6	507 9.50	1.0	5.7	1.9	2.9
6	97.6 101.7	--	125[1] 6.03	209 11.5	313 27.2	4.8	5.5	8.7	6.3
7	101 105.2	6.26	145 10.8	298 15.6	504 46.5	7.4	5.2	9.2	7.3
8	100.0 104.2	55.0	182 16.2	346 14.0	555 33.5	8.9	4.0	6.0	6.3
9	99.2 103.3	86.3	169 17	377 1.27	582 98.6	10.1	0.34	16.9	9.1
10	101 105.2	13.6	151 45.5	286 15.7	409 77.7	30.1	5.5	19.0	18.2
11	97.2 101.3	--	149 3.06	297 3.61	485 2.52	2.1	1.2	0.52	1.3
12	84.7[1] 88.2	--	164 10.1	315 4.6	578 26.0	6.2	1.5	4.5	4.1
Mean	97.5	27.5	161	298	489				
Mean (%)	101.6		89.0[2]	90.2[2]	92.3[2]				
Std. Dev.	3.97		12.2	49.3	88.3				
Coef. Var.(%)	4.1		7.6	16.5	18.1				

[1] Outlier

[2] Mean Corrected for Blank

MICRO METHOD FOR THE DETERMINATION OF CHLORINATED PESTICIDES IN HUMAN OR ANIMAL TISSUE AND HUMAN MILK

I. INTRODUCTION:

The size of many tissue samples is so minimal that the method described in Section 5,A,(1) is unsuitable. This procedure, requiring only 0.5 grams, is suitable for most biopsy samples and for wildlife (small animal or bird) tissues.

REFERENCE: Presentation at Fall meeting, ACS, Chicago, IL, 1967 MICROMODIFICATION OF THE MILLS PROCEDURE FOR THE DETECTION OF PESTICIDES IN HUMAN TISSUES. Enos, H. F., Biros, F. J., Gardner, D. T., Wood, J. P.

II. PRINCIPLE:

A 0.5 gram sample of tissue is macerated in a micro tissue grinder with acetonitrile. An aqueous solution of Na_2SO_4 is added, the pesticides are partitioned into hexane and the extract is concentrated to 0.3 ml. Cleanup and partitioning are carried out by successive elutions with 1% methanol in hexane through a micro column of Florisil. Two fractions are collected, concentrated to suitable volumes by evaporation in a modified micro Snyder assembly, and subjected to GLC with electron capture detection.

III. MATERIALS AND REAGENTS:

1. MICROCOLUMN:

Place a small loose plug of glass wool in the tip of a size "B" Chromaflex column. (Kontes Cat. No. 42100, Size 22-7 mm) Pack the column with 1.6 gm of 60 to 100 mesh Florisil which has been activated by the manufacturer at 1200°F. (Only PR grade Florisil should be used for this method.) The column packing is added in increments followed by a gentle tapping. Add 1.6 gm of sodium sulfate, granular, to the top of the column. Wash the column with 50 ml of Nanograde hexane followed by 50 ml of Nanograde methanol. Dry and store columns in a 130°C oven. The columns should be conditioned at 130°C at least overnight before using. For routine work it is convenient to prepare a large number of columns at one time.

2. SODIUM SULFATE, ANHYDROUS, GRANULAR:

   Store in glass stoppered bottles in an oven at 130°C. Extract a portion of the sodium sulfate, equivalent to the amount used in the Florisil column, with hexane. Concentrate the extract to 50 µl and inject 5 µl into the gas chromatograph. The results will indicate whether it is necessary to extract the batch of sodium sulfate with hexane and methanol prior to storing in the oven.

3. PESTICIDE QUALITY SOLVENTS:

   Hexane, acetonitrile, methanol.

4. DISTILLED WATER:

   Extract 8.0 ml with hexane. Concentrate the extract to 300 µl, and inject 5 µl into the gas chromatograph. If extraneous peaks occur, then the distilled water must be extracted with hexane prior to use.

5. TISSUE GRINDER:

   Dual tissue grinder Size 22 or 23 (Kontes Cat. No. K-885450).

6. MIXER:

   Vortex Junior or equivalent.

7. CENTRIFUGE:

   Capable of a speed of 2,000 rpm

8. EVAPORATIVE CONCENTRATOR:

   Complete with modified micro Snyder column, Ŧ joint 19/22, Kintes Cat. No. K-569250.

9. CONCENTRATOR TUBE:

   Size 1025, Kontes Cat. No. K-570050.

10. CONCENTRATOR TUBE:

    Size 2525, Special Order, Kontes Cat. No. K-570050.

11. TEST TUBE:

    25 ml with $ 19/22, joint with hooks, Special Order, Kontes Cat. No. K-897900.

IV. SAMPLE PREPARATION - LIVER, KIDNEY, BONE MARROW, ADRENAL, GONADS:

1. Extract a 500-mg sample of tissue in a size 22 or 23 dual tissue grinder with 2.5 ml of acetonitrile. Add 20 nanograms of aldrin, in 0.1 ml of hexane, to the tissue grinder. This will serve as a recovery check as well as a marker for relative retention time.

   NOTE: Run a complete reagent blank with each set of samples.

2. Centrifuge and pour supernatant into a 50-ml round bottom test tube. Repeat extraction twice more, collecting supernates in the test tube.

3. Add 25 ml of 2% aqueous sodium sulfate to the test tube and mix the contents with the aid of a Vortex mixer.

4. Extract the aqueous acetonitrile mixture with one 5-ml and two 2-ml portions of hexane. Combine the extracts in a 10-ml evaporative concentrator.

5. Concentrate the extract to 300 μl with the aid of a modified micro Snyder column* and a 3-mm glass bead in the tube.

6. Proceed to Subsection V.

V. FLORISIL FRACTIONATION:

1. Remove a Florisil column from the oven and allow it to cool to room temperature.

2. Pre-wet the column with 10 ml of hexane and discard the eluate.

3. Transfer the 0.3 ml of extract remaining after step (5) in Subsection IV, to the top of the Florisil column with the aid of a disposable pipet fitted with a rubber bulb. Begin immediate collection of eluate in a 25-ml capacity concentrator tube.

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*J. Burke et al., J.A.O.A.C., 49 (5): 999-1033, 1966.

4. Rinse the 10-ml concentrator tube with 0.25 ml of hexane transferring this to the top of the column. Repeat this step a second time.

5. Proceed with the elution and collection using a total of 12 ml of hexane followed by 12 ml of 1% methanol in hexane. This 24 ml represents fraction one, and will contain heptachlor, aldrin, *p*,*p*'-DDE, *o*,*p*'-DDT, and *p*,*p*'-DDT.

6. Collect a second fraction by eluting with a second 12 ml portion of 1% methanol in hexane. This fraction will contain dieldrin, heptachlor epoxide, endrin, β-BHC, Lindane, and *p*,*p*'-DDD. (See Table 1.)

   NOTE: A small amount of β-BHC, Lindane, and/or *p*,*p*'-DDD may appear in the first fraction.

7. Add 20 nanograms of aldrin in 0.1 ml of hexane to fraction two, evaporate both fractions using a modified micro Snyder column and a 3 mm glass bead in the tube.

8. Adjust the volumes in fractions (1) and (2) to 500 and 300 µl, respectively, and proceed with the GLC portion as outlined in Subsection VII.

VI. ANALYSIS OF BRAIN:

Proceed with steps (1) through (4) as described under IV. SAMPLE PREPARATION.

5. Concentrate the combined hexane extracts to 500 µl in a 25 ml test tube fitted with a modified micro Snyder column and using a 3 mm glass bead in the tube.

6. Add 0.3 ml Acetic Anhydride and 0.3 ml pyridine and incubate in a water bath at 60 to 65°C for 1/2 hour.

7. Add 9 ml of 2% Na_2SO_4 and extract with 2 to 3 ml portions of hexane.

8. Concentrate the combined extracts to 300 µl in a 10 ml evaporative concentrator fitted with a modified micro Snyder column using a glass bead for a boiling chip.

9. Proceed as described under V. FLORISIL FRACTIONATION.

VII. ANALYSIS OF HUMAN MILK:

The basics of this procedure have been determined by experience in a laboratory conducting intensive surveillance to be wholly applicable to the analysis of human mother's milk. A few modifications have proved critical, however, and these are given in the following:

1. Follow Subsection IV, all steps as described but with one precautionary comment. If the sample has been frozen, it has been found advisable to use a supersonic disintegrator to homogenize it after thawing.

2. Unlike cow's milk, no curd layer has been observed forming on top; instead, there is sediment at the bottom of the tissue grinder with generally a thin aqueous layer between it and the solvent layer. The solvent layer is pipeted after the first extraction, and the second extraction usually gives a homogeneous liquid.

3. In countries where the use of DDT is permitted by law, the chemist may find it advisable to dilute the final extract to 1.0 ml or greater instead of a final volume of 300 µl as specified in the final step.

VIII. GAS LIQUID CHROMATOGRAPHY:

Proceed with electron capture gas chromatography following the general guidelines set forth in Section 4,A,(4) and making sure that prevalent system sensitivity complies with the criteria given in Misc. Note in Section 4,A,(4).

TABLE 1. ELUTION PATTERN OF SOME COMMON CHLORINATED AND ORGANOPHOSPHORUS PESTICIDES ON MICRO FLORISIL COLUMN.

Compound	12 ml hexane + 12 ml 1% methanol in hexane Fraction I	Additional 12 ml 1% methanol in hexane Fraction II
Aldrin	x	
α-BHC	x	x
β-BHC		x
γ-BHC	x	x
δ-BHC		x
DDA, methyl ester		x
o,p'-DDD	x	x
p,p'-DDD	x	x
o,p'-DDE	x	
p,p'-DDE	x	
o,p'-DDT	x	
p,p'-DDT	x	
Diazinon	x	x
Dieldrin		x
Endosulfan I & II		x
Endrin		x
Ethion		x
Ethyl parathion		x
Heptachlor	x	
Hept. epoxide		x
1-Hydroxychlordene		x
Malathion		x
Methyl parathion		x
Methoxychlor		x
Nitrofen	x	x
Paradichlorobenzophenone		x
Polychlorinated biphenyls	x	
Ronnel		x
Toxaphene	x	x

☆ ANALYSIS OF HUMAN BLOOD OR SERUM

I. INTRODUCTION:

Because of its availability and probable diagnostic value with regard to extent of both chronic and acute exposures to chlorinated hydrocarbon and other classes of pesticides, blood specimens present a convenient tissue for study, providing meaningful data pertinent to the Community Study and Monitoring laboratory program. Of several methods available in the literature, the Dale et al. (1966) method provided some desirable features in rapidity, simplicity and sensitivity for the determination of chlorinated insecticides and related materials in blood. The Dale et al. method, as published, was found to yield poor precision between laboratories, and in fact, between chemists within a laboratory. However, a method including these features is essential in the monitoring situation involving analyses of large numbers of samples. The following procedure utilizes only the direct solvent extraction principle of the Dale et al. method. It is to be considered a general survey method for the determination of chlorinated hydrocarbon pesticide levels in blood, particularly DDT and its metabolites. For an in-depth study of total pesticide residue levels in this tissue, it is recommended that a cleanup method for the determination of chlorinated pesticides in human tissue, (i.e., Section 5,A,(1) in this manual) be applied, together with confirmatory determination such as TLC and chemical derivatization techniques.

REFERENCE: Dale, W. E., A. Curley, and C. Cueto, (1966). Hexane Extractable Chlorinated Insecticides in Human Blood, Life Sciences 5: 47.

II. PRINCIPLE:

A 2-ml aliquot of serum is extracted with 6 ml of hexane in a round-bottom tube. The extraction is conducted for 2 hours on a slow-speed rotating mixer. The formation of emulsion is unlikely, but if it should occur, centrifugation may be used to effect separation of the layers. A 5-ml aliquot of the hexane layer is quantitatively transferred to an evaporative concentrator tube to which is affixed a modified micro-Snyder column. The extract is concentrated in a water or steam bath, and the final volume is adjusted to correspond to the expected concentration of the pesticide residue. A suitable aliquot is analyzed by electron capture gas chromatography.

III. APPARATUS AND REAGENTS:

1. A rotary mixer so designed as to accommodate the 16 mm culture tubes and which may be operated at a rotary speed of 50 rpm. Fisher Scientific Company, Roto-Rack™, Cat. No. 14-456.
2. Gas chromatograph fitted with electron capture detector. Recommended GLC columns and operating parameters are given in Section 4,A.
3. Tubes, Culture, 16 x 125 mm, fitted with screw caps, size 15-415 with Teflon-faced rubber liners, Corning No. 9826.
4. Micro-Snyder column modified, with 19/22 ₮ joint, Kontes No. K-569251.
5. Concentrator tube, 10 ml, grad. 0 to 1 x 0.1 and 2 to 10 x 1, 19/22 ₮ joint, size 1025, Kontes No. K-570050.
6. Syringe, 100 μl, Hamilton No. 710 or equivalent.
7. Vortex Genie mixer.
8. Pipet, Mohr type, 1 ml grad. in 0.01 ml increments. Corning No. 7063 or equivalent.
9. Pipets, transfer, 2, 5, and 6 ml Corning No. 7100 or the equivalent.
10. Beads, solid, glass, 3 mm, Corning No. 7268 or the equivalent.
11. Six-place tube carrier, stnls. steel. May be fabricated at local tin shop per attached sketch.
12. Water bath capable of holding temp. of 95 to 100°C.
13. Centrifuge with head to accommodate the Corning No. 9826 tube, capable of speed of 2,000 rpm.
14. Hexane, distilled in glass, pesticide grade.

IV. SAMPLING:

After drawing sample from the donor (7 to 10 ml), it should be transferred to a vial or tube fitted with a Teflon or foil lined screw cap. DO NOT USE CAPS OF POLYETHYLENE OR RUBBER.

Place whole blood sample in the refrigerator for about 30 minutes for a settling period and then centrifuge for a sufficient time for the separation of at least 3 ml of clear serum - generally 10 minutes at 2,500 rpm. Whether or not the analysis is to be conducted immediately, it is desirable at this point to transfer the 2 ml sample aliquot to the 16 x 125 mm culture tube used for extraction. If analysis cannot be run immediately, place in refrigerator at 2-5°C for periods of up to 24 hours before analysis. If time interval to analysis exceeds 24 hours, the tube should be stored in a deep freeze at -15 to -25°C. Stored in this manner, analysis may be delayed for periods up to a month without undue effects on the chlorinated pesticides present.

V. PROCEDURE:

1. Mix blood serum sample thoroughly and, with a volumetric pipet, transfer 2 ml to a 15 ml round bottom culture tube.

   NOTE: In case of the presence of a flocculent or sedimentary material, it is strongly recommended that the sample be centrifuged ca 5 minutes @ 2,000 rpm before pipetting the 2 ml aliquot. Failure to observe this point may result in poor reproducibility of replicated analyses of the same sample.

2. Add 6 ml hexane from a volumetric pipet. Tightly stopper the culture tube with a Teflon-lined screw cap. Place tube on rotator.

3. Set rotator speed at 50 rpm and rotate for 2 hours.

   NOTES: (1) This speed may vary from 50 to 44 rpm but should be confined to this range.

   (2) Unless the sample is extremely old, emulsion formation should present no problem. In case it occurs, centrifuge at 2,000 rpm 4 to 5 minutes, or longer if necessary, to effect sufficient separation to permit withdrawal of the 5 ml aliquot of clear extract.

4. With a volumetric pipet, transfer 5 ml of the hexane extract to a 10 ml grad. concentrator tube, add one 3 mm glass bead, and attach a modified micro-Snyder column. Evaporate the extract in a steam or hot water bath at 100°C to a volume slightly less than that which is estimated as appropriate to accommodate (1) the current level of electron capture detector sensitivity, and (2) the expected residue range in the

particular sample. When working with general population blood of low pesticide levels, it may be necessary to evaporate to ca 0.5 ml.

NOTES: (1) With some experience the operator can complete the evaporation step in less than 5 minutes. The tube must be withdrawn from the water when boiling agitation becomes too vigorous. Immersion and withdrawal are alternated based on observation of boil agitation.

(2) Up to six tubes of extract may be evaporated simultaneously by using the special rack shown in Figure 2. Time and motion studies have shown that the time required for the evaporation period is equal to that required for a single tube.

(3) When working with blood from high exposure donors, the 5-ml aliquot may require dilution rather than concentration. This can be determined by a preliminary analysis of the 5-ml aliquot.

(4) With lower concentrations, use higher degree of concentration samples.

5. Allow the tube to cool (3 to 5 minutes), remove the micro-Snyder column, and rinse down the sides of the tube and the column joint with hexane. The volume used will depend on the desired dilution.

NOTES: (1) When a minimal dilution is required after evaporation, a 100-μl syringe is useful in performing the hexane rinse.

(2) To obtain a suitable extract concentration for p,p'-DDE, it is generally necessary to adjust the extract volume to a level in excess of 1 ml. In this case, add hexane until the meniscus is exactly at the 1-ml mark on the concentrator tube. Then use a 1-ml Mohr pipet for total volumes up to 3 ml.

For larger volumes, use a 5-ml Mohr pipet, carefully measuring the volume of hexane delivered. Above the 1 ml graduation mark, the concentrator tube calibrations are not sufficiently accurate for use in this analysis.

It is also good practice to check the graduation marks up to 1 ml for all concentrator tubes used in this analysis.

6. Stopper the concentrator tube and hold on the Vortex mixer, set for high speed for ca 30 seconds for volumes of 6 ml or less. It is safer practice to mix a full minute for larger volumes.

7. Proceed with electron capture GLC observing the guidelines set forth in Section 4,A,(4).

VI. CALCULATIONS:

The following equation is applicable when all volumes specified in the method are followed precisely, with no exceptions:

$$\text{ppb} = \frac{a\ b\ x}{c\ y} \times 0.6$$

Where
a = nanograms of pesticide in standard peak
b = height of sample peak
c = height of standard peak
x = total volume of final extract in microliters
y = microliters of extract injected

Example:
nanograms in standard peak = 0.3
height of sample peak = 80 mm
height of standard peak = 90 mm
total volume of final extract = 1,000 μl
volume of final extract injected = 5 μl

$$\text{ppb} = \frac{0.3 \times 80 \times 1000}{90 \times 5} = 0.6 = 32 \text{ ppb}$$

SPECIAL NOTE:

All analytical research and subsequent collaborative study of the method was conducted with each laboratory following the procedure exactly as written. In all probability, a serum sample of less than 2 ml can be analyzed with confidence, provided the same serum to hexane ratio is followed. The precision resulting from the use of reduced volumes is not known, however. If such deviation must be made, the final calculation may be accomplished by using the following basic equation:

$$\text{ppb} = \frac{a\ b\ e}{c\ d}$$

Where a, b, and c are the same as given for the simplified equation
d = ml (or grams) in original sample
e = dilution factor obtained as follows:

$$\frac{\text{ml of hexane added to serum X final extract volume } (\mu l)}{\text{aliquot volume of extract (ml) X } \mu l \text{ injected}}$$

Example: Assuming that the same serum used to illustrate the simplified equation was available in a volume less than 2 ml.

nanograms in standard peak	=	0.3
height of sample peak	=	61.5 mm
height of standard peak	=	90 mm
ml of serum in original sample	=	1.6
ml of hexane added to serum		5
final extract volume	=	1,000 μl
volume of extract aliquot	=	4 ml
injection volume	=	5 μl

$$\text{dilution factor (e)} = \frac{5 \times 1{,}000}{4 \times 5} = 250$$

$$\text{ppb} = \frac{0.3 \times 61.5 \times 250}{90 \times 1.6} = 32 \text{ ppb}$$

VII. REPORTING LIMITS - DETECTABILITY:

The Analytical Chemistry Committee has established the following minimum reporting limits for chlorinated pesticides in serum:

β-BHC, lindane, aldrin, heptachlor, heptachlor epoxide, o,p'-DDE, p,p'-DDE, dieldrin---------1 part per billion.
Endrin, o,p'-DDT, p,p'-DDD, p,p'-DDT-2 parts per billion.

If chromatographic peaks indicate the presence of any compound in a quantity less than the minimum reporting level, the compound shall be reported as trace (TR).

VIII. APPLICATION OF MILLS, ONLEY, GAITHER CLEANUP TO SERUM:

Some laboratories may wish to pool sera for Florisil cleanup and an in-depth appraisal of the pesticides present. When this is indicated, the following steps are taken:

1. Measure 50 ml of serum into a 1-L sep. funnel containing 190 ml of CH_3CN, 200 ml of aqueous 2% Na_2SO_4 and 50 ml of hexane.

2. Stopper, shake funnel vigorously 2 minutes, and allow the layers to separate.

3. Draw off the aqueous (lower) layer into a second 1-L sep. funnel and percolate the hexane layer through a 2-in. column of anhydrous Na_2SO_4 into a 500-ml Kuderna-Danish flask fitted with a 10-ml grad., evap. concentrator tube containing one 3-mm glass bead.

4. Add another 50-ml portion of hexane to the aqueous solution in the second 1-L separator; stopper and shake vigorously another 2 minutes. When layers have separated, draw aqueous layer back into the first 1-L separator and percolate the hexane layer through the Na_2SO_4 into the K-D flask. Repeat the extraction twice more resulting in a total hexane extract of 200 ml.

5. Assemble K-D evaporator and concentrate extract to ca 3 ml. Disassemble evaporator, rinsing tube joint with a small volume of hexane, and dilute extract to exactly 5 ml. Stopper and shake on Vortex mixer 2 minutes.

6. From this point on, follow the procedure outlined in Section 5,A,(1) starting with Subsection VIII, Step 1 and following through precisely as outlined.

FIGURE 1. ROTO-RACK^R Mixer, variable speed

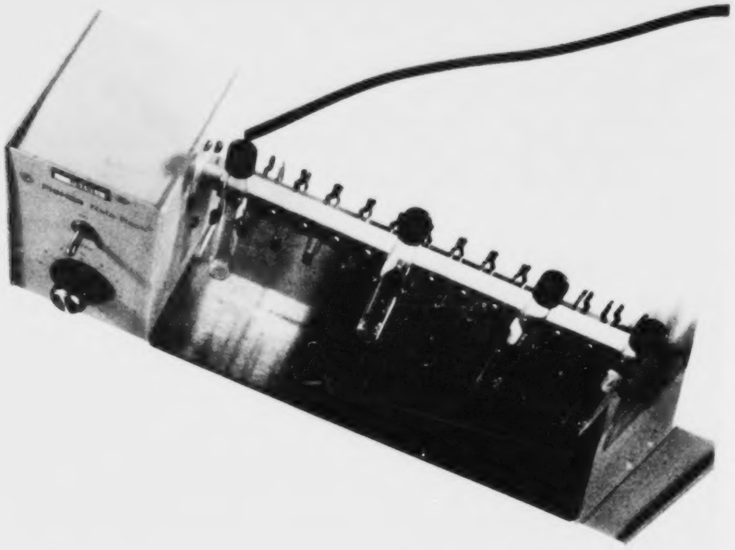
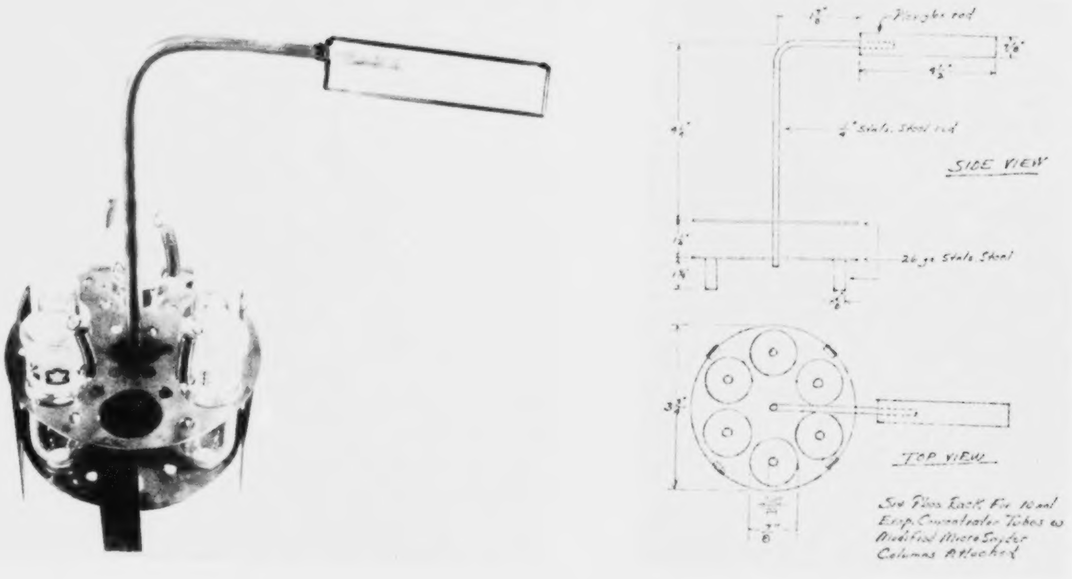


FIGURE 2. Evaporative concentrator tube holder, 6-place, stainless steel


1 7/8"
Plexiglas rod
7/8"
4 1/2"
4 1/4"
1/4" Stnls. Steel rod
SIDE VIEW
1 1/2"
26 ga Stnls. Steel
1 1/8"
3 3/4"
TOP VIEW
Six Place Rack For 10 ml
Evap. Concentrator Tubes w
Modified Micro Snyder
Columns Attached

DETERMINATION OF PENTACHLOROPHENOL (RAPID METHOD) IN BLOOD

I. INTRODUCTION:

Pentachlorophenol (PCP) is an herbicide, defoliant, and antimicrobic chemical used throughout the United States as a preservative agent for many products. Pentachlorophenol seems to be present everywhere, appearing in municipal water supplies, wells, paints, wood and paper products, and in blood and urine of every person now being examined. The ubiquity of human exposure to this potentially dangerous compound has prompted concern in the field of public health. This interest has been stimulated by several recent industrial and public intoxications which resulted in fatalities.

The method described herein incorporates portions of a method currently in review by Rivers, and portions from a method by Cranmer and Freal for PCP in urine.

REFERENCES:

1. Rivers, J. B., Gas Chromatographic Determination of PCP in Human Blood and Urine, Bull. of Envir. Contam. & Toxicology, Vol. 8, No. 5, 294-296, 1972.

2. Cranmer, M., and Freal, J., Gas Chromatographic Analysis of Pentachlorophenol in Human Urine by Formation of Alkyl Ethers, Life Sciences, Vol. 9., Part II, pp 121-128, 1970.

II. PRINCIPLES:

A rapid method is described for the determination of PCP based on its conversion to a methyl ether after a 2-hour extraction of the acidified sample in benzene. EC GLC is utilized for quantitation, comparing sample peak against peaks from known standards, similarly methylated.

III. APPARATUS:

1. Gas chromatograph with EC detection, fitted with either or both columns of 4% SE-30/6% QF-1 and 5% OV-210. The 1.5% OV-17/1.95% QF-1 should not be used.

2. Rotary mixing device, "Roto-Rack™", Fisher Scientific Company, No. 14-057.

3. Tubes, culture, 16 x 125 mm, fitted with screw caps, Size 15-415 with Teflon-faced rubber liners, Corning No. 9826.

4. Pipets, transfer, 2, 3 and 6 ml, Corning No. 7100 or the equivalent.

5. Pipets, Mohr type, 0.5 ml grad. in 0.01 ml, Corning No. 7063 or the equivalent.

6. Flasks, vol., 10 ml.

7. Centrifuge, capable of spin velocity of 2000 rpm.

8. Vortex mixer.

IV. REAGENTS AND SOLVENTS:

1. Benzene, pesticide quality.

2. Hexane, pesticide quality.

3. Methanol, pesticide quality.

4. 2, 2, 4-Trimethylpentane, pesticide quality.

5. Acid, sulfuric, conc., reag. grade.

6. N-Methyl-N'-nitroso-N-nitrosoguanidine, Aldrich Chemical Co., Inc., Milwaukee, WI.

7. Diazomethane methylating reagent:
Add 5 ml of 20% aqueous sodium hydroxide to a 15 ml test tube. Place a volume of hexane, in excess of that to be used and not less than 3 ml, on the 20% sodium hydroxide solution. Slowly add N-methyl-N'-nitro-N-nitrosoguanidine reagent to the hexane in approximately 1 mg increments until a saturated hexane-diazomethane solution, indicated by a constant yellow color, is obtained. The reagent is ready for use only after diazoalkane gas is no longer evolved.

   NOTE: Use extreme caution when handling the skin irritant diazoalkane reagent since both the reagent and the diazoalkane gases are extremely toxic, carcinogenic and potentially explosive. Diazoalkane generation should be carried out in a high draft hood. Use of safety goggles and disposable gloves is desirable and close adherence to manufacturers' recommendations for storage and handling is strongly recommended. Diazoalkane solutions should not be pipetted by mouth. It is suggested that diazoalkane solution be prepared fresh, as materials resulting in interfering peaks appear during storage. The volume prepared should not be greatly in excess of that required. The original hexane-diazoalkane generating solution should not be stored in ground glass stoppered containers nor in bottles with visible interrior etching; however, no hazard is involved in the culture tubes containing the PCP benzene extract plus diazoalkane. Extended exposure to air destroys the diazoalkane reagents.

8. Pentachlorophenol, analytical standard. Available from Reference Standards Repository at Research Triangle Park, NC.

9. Preparation of Standard Solutions.
Dissolve 10 mg of PCP in 100 ml of benzene. Dilute 1 ml of this solution to 100 ml with hexane. The resulting stock solution has a concentration of 1 ng/μl.

   React a 1-ml aliquot of the diluted stock solution with 0.25 ml of the diazomethane reagent as described under Methylation. The solution resulting from the derivatization reaction contains 800 pg of PCP per μl. Larger volumes may be used but strict adherence to this ratio of the 1 ng/μl solution to alkylating reagent should be maintained. The working standards, in a range of 10 to 30 pg/μl, are prepared by diluting the derivatized stock with isooctane.

V. SAMPLING:

Extreme care and precautionary measures should be taken to insure freedom of the sample of contamination. The reader is advised to carefully review the comments offered in the SAMPLING Subsection IV of Method 5,A,(4),(a) pertaining to urine analysis for PCP.

VI. PROCEDURE:

Extraction

1. In a 16 x 125 culture tube, combine 2 ml of blood serum and 6 ml of benzene.

NOTE: Because of the widespread prevalence of PCP, a reagent blank consisting of 2 ml of pre-extracted distilled water (Subsection VI,4.) should be carried through the entire procedure along with the sample(s).

2. Add 2 drops of conc. H_2SO_4, seal tightly with Teflon-lined screw cap, and rotate for 2 hours at 50 rpm on the "Roto-Rack".

   NOTE: If, after the extraction period, the layers do not separate completely, centrifuge 5 minutes at 2,000 rpm.

3. Transfer 3 ml of the benzene (upper) layer to a 10-ml vol. flask and proceed with methylation.

Methylation

1. Add 0.3 ml of the methylating reagent (IV.,7.), stopper flask and mix on Vortex for 2 minutes.

2. Allow to stand for 20 minutes and dilute to 10 ml volume with isooctane or hexane.

3. Make an initial injection into the gas chromatograph of 5 µl to determine the degree of dilution that may be required to obtain peaks within 25% of the peak height response from one of the working standards.

VII. MISCELLANEOUS NOTES:

1. Recovery studies by the author given in Table 1 indicated recoveries over 90% for PCP concentrations of 190 ppb and higher. The stated lower limit of detection is 10 ppb.

2. The method outlined here is relatively simple and rapid, and utilizes equipment most of the laboratories have on hand. In areas where the general population is continuously exposed to PCP (for example, in Dade County (Miami), from framing of all dwellings), blood serum levels in excess of 100 ppb are not uncommon.

   Little or no information is available concerning the levels prevalent in the general population of the northern tier of states in the U. S. where exposure to PCP should be far less than that in the sub-tropical areas. Therefore, it cannot be predicted at this time whether general population blood in these northern areas might contain PCP residues approaching or less than the stated minimum detectability of this method. Should this prove to be the case in any laboratory, a modification of the method contained in this manual for PCP in urine [5,A,(4),(a)] might prove more suitable than the method described here. That method, using a larger initial sample, also incorporates a partitioning for removal of a portion of the contaminants. Furthermore, using hexane as the extracting solvent, it seems probable that less extraneous materials would be extracted.

3. Use of the 1.5% OV-17/1.95% QF-1 column is not recommended in this determination. On this column the relative retention value for 2,4-D, methyl ester is identical to that of PCP (methyl ester). Therefore, if the sample should contain 2,4-D and/or PCP and 2,4-D, resolution by GLC would not be possible.

   This should pose no problem on the other two columns used in the program as the RR values at 200°C are:

	SE-30/QF-1	OV-210
2,4-D(ME)	0.44	0.09
PCP (ME)	0.63	0.56

4. All reagents including the distilled water used in the method must be extracted with hexane before use as they may be contaminated with PCP or other materials which may cause interferences. Glassware should be washed with dilute NaOH solution followed by deionized water and acetone rinse. Care should be taken not to permit contact between wooden or paper materials and glassware, as peg boards and some brands of absorbent paper products have been found to contain PCP.

5. If the recommended volumes are used, calculations are simplified and are as follows:

   PCP(in ppb) in serum = pg/μl injected times 10.

TABLE 1. PERCENT RECOVERY OF PCP FROM SAMPLES FORTIFIED BEFORE EXTRACTION

Sample	PCP Found, ppm[a]	PCP Added, ppm	PCP Recovered, ppm	Recovery %
Blood Plasma	0.19	0.50	0.67	96
			0.65	92
			0.68	98
		5.00	4.58	88
			4.70	90
			4.70	90
			4.70	90
		5.0	46.9	93
			48.5	97
			42.0	84
				Mean 92[b]

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[a] Limit of detectability 0.01 ppm

[b] Standard deviation ±4.5%

PENTACHLOROPHENOL (PCP) AND CHLORINATED PHENOL METABOLITES OF PCP AND HCB

I. INTRODUCTION:

Pentachlorophenol (PCP) and its sodium and copper salts are well known wood dressings, aquatic and terrestrial herbicides, and antimicrobials used extensively throughout the United States. PCP seems to be present everywhere, appearing in numicipal water supplies, wells, paints, wood, and paper products. Human exposure may occur through several routes, including inhalation of dusts, dermal absorption of powders and solutions, and ingestion of residues present in food and water. Due to the aqeous solubility of PCP salts, human elimination occurs, at least in part, through the urinary system, providing a convenient monitor for PCP exposure.

The following method for the determination of PCP in urine includes a hydrolysis step that gives a much higher level for biologically incorporated PCP than the previous method in this section not specifying hydrolysis. The new procedure is highly selective and more quantitative, and allows determination of PCP at low parts per billion levels. Also described below is the multiresidue quantitation and confirmation of several chlorinated phenol metabolites of PCP and the chlorinated insecticide hexachlorobenzene (HCB).

The major metabolites from an HCB feeding study were identified as PCP, tetrachlorohydroquinone, and pentachlorothiophenol. The major metabolite of PCP was tetrachlorohydroquinone and a minor metabolite was tetrachloropyrocatechol. Based on these results, pentachlorothiophenol in urine can be used as an indicator of possible exposure to HCB: PCP exposure would be indicated by a high level of PCP and the presence of tetrachlorohydroquinone and tetrachloropyrocatechol in urine.

The analytical methods have been tested on fortified urine samples, rat urine, human general population urine, and urine from a worker occupationally exposed to PCP.

REFERENCES:

1. Determination of Pentachlorophenol in Urine: The Importance of Hydrolysis, Edgerton, T. R., and Moseman, R. F., J. Agr. Food Chem. 27, 197 (1979).

2. Multiresidue Method for the Determination of Chlorinated Phenol Metabolites in Urine, Edgerton, T. R., Moseman, R. F., Linder, R. E., and Wright, L. H., J. Chromatogr. 170, 331 (1979).

II. PRINCIPLE:

PCP and chlorinated phenol metabolites of PCP and HCB are extracted with benzene after acidification of urine and hydrolysis. The phenols are methylated with diazomethane before electron capture gas chromatography. Cleanup and separation of methylated phenols into groups is carried out on an acid alumina column. This step is essential for determination at low ppb levels.

Compounds are confirmed by GLC-MS.

III. APPARATUS AND REAGENTS:

1. Tracor MT-220 gas chromatograph equipped with a ^{63}Ni pulsed linearized mode electron capture detector, or equivalent, operated with the parameters given in Section VIII.

2. Anhydrous, granular sodium sulfate and sodium bisulfite, Soxhlet extracted for 4 hours with hexane and oven dried at 130°C.

3. Acid alumina, Brockmann Activity I, Fisher Scientific Co., dried for 24 hours at 130°C and stored in a desiccator.

4. Potassium hydroxide and hydrochloric acid, reagent grade.

5. Benzene, diethyl ether, acetone, and hexane, pesticide grade or equivalent.

6. N-Methyl-N'-nitro-N-nitrosoguandine (diazomethane), Aldrich Chemical Co. CAUTION! This compound is a known carcinogen.

7. Preparation of methylating reagent:

   a. Dissolve 2.3 grams of potassium hydroxide in 2.3 ml of distilled water in a 125 ml Erlenmeyer flask and cool to room temperature.

   b. Add 25 ml of diethyl ether and cool the flask in the refrigerator.

   c. In a glovebox or high draft hood, add 1.5 grams of N-methyl-N'-nitro-N-nitrosoguanidine in small portions to the flask with vigorous shaking.

d. Decant the ether layer into a scintillation vial and store in a freezer.

NOTE: Use EXTREME CAUTION when handling the skin irritant diazoalkane reagent because both the reagent and the diazolkane gases are extremely toxic, carcinogenic, and potentially explosive. Diazoalkane generation should be carried out in a high draft hood. Use of safety goggles and disposable gloves is desirable, and close adherence to manufacturer's recommendations for storage and handling is strongly recommended. Diazoalkane solutions should not be pipetted by mouth. It is suggested that diazoalkane solution be prepared fresh, as materials resulting in interfering peaks appear after storage. Extended exposure to air destroys the diazoalkane reagents.

8. Pentachlorophenol (99+%), 2,3,4,6-tetrachlorophenol; 2,3,5,6-tetrachlorophenol; pentachlorothiophenol; 2,3,4,5-tetrachlorophenol, Aldrich Chemical Co. tetrachloropyrocatechol, Pfaltz and Bauer. Tetrachlorohydroquinone, K and K Laboratories. Recrystallize pentachlorothiophenol, tetrachlorohydroquinone, and tetrachloropyrocatechol before use.

9. Preparation of PCP and other phenol standard solutions:

   a. Prepare an analytical standard of 200 μg/ml for each phenol in hexane and store at -15°C in a brown glass bottle.

   b. Pipet a volume containing 10 μg of each phenol into separate 15 ml graduated centrifuge tubes.

   c. Methylate the solutions of the phenols by adding, in a high draft hood, 5 ml of diazomethane reagent (item 7 above) to each tube.

   d. Let the phenol standards stand for 1 hour.

   e. Bubble nitrogen through the individual standard solutions to remove any excess diazomethane.

   f. Dilute the solution to the proper concentration for direct EC GLC or subject to acid alumina column cleanup before EC GLC.

NOTES:

1. A known amount of each phenol can be methylated as a mixture rather than reacting the individual compounds. The mixture is also allowed to stand one hour before EC GLC determination.

2. Make urine fortifications from acetone dilutions of the seven mixed phenol standards.

10. Glass wool.

11. Erlenmeyer flask, 125 ml.

12. Scintillation vial.

13. Culture tube, Teflon-lined screw-cap, 20 x 125 mm.

14. Centrifuge tubes, 15 ml.

15. Mechanical rotator.

16. Pipets, disposable Pasteur.

17. Pipets, volumetric.

18. Centrifuge.

19. Apparatus for concentration of solutions by nitrogen blow-down, including water bath operated at 30°C.

20. Chromaflex column, size 22-9, Kontes 420530.

IV. SAMPLING:

It is mandatory that extreme care be taken in the preparation of the glass containers and caps used to hold the sample and the manner in which the sample is taken. Pentachlorophenol is very prevalent in the environment, to such an extent that many commonplace materials may contain levels sufficiently high to grossly contaminate a sample. Paper products and wood frequently contain the compound, most particularly in subtropical and tropical areas where pressure treated lumber is widely used in construction.

All sample containers must be scrupulously prepared by first washing, then soaking in dilute NaOH followed by rinses with deionized water and acetone. During drying, the interiors of bottles and caps should be protected from air dust contamination, and must not be allowed to contact wood or paper surfaces.

All bottle caps should be Teflon- or aluminum foil-lined. Under no circumstances should the paper liner of the bottle cap be allowed to come in contact with the sample. Paper-lined caps may be used only if a layer of foil or Teflon is inserted to isolate the sample from the paper liner.

V. EXTRACTION OF URINE:

NOTE: Before starting the analysis, the chemist should make certain that all glassware used in the analysis has been specially prepared as described in Section XIII,1.

1. Transfer 2 ml of urine to a Teflon-lined screw cap culture tube.

2. Add 100 mg of sodium bisulfite.

NOTE: Bisulfite is added to urine samples before hydrolysis and to urine extracts after hydrolysis to act as a reducing agent (see Subsection IX,2).

3. Acidify with 0.5 ml of concentrated hydrochloric acid.

4. Seal the tube and place in a boiling water bath for 1 hour with periodic shaking to achieve hydrolysis.

NOTE: A hydrolysis time of 1 hour is necessary for the maximum freeing of conjugated PCP in urine. Further hydrolysis does not yield additional PCP.

5. Remove the tube and cool to room temperature.

6. Add an additional 100 mg of sodium bisulfite.

7. Extract the sample with 5 ml of benzene for 1 hour on a mechanical rotator at 30-50 rpm.

8. Centrifuge the solution and transfer the benzene layer to an aluminum foil-wrapped 15 ml centrifuge tube with a disposable pipet.

NOTE: Wrapping with aluminum foil minimizes the possible effects of photodecomposition.

9. Repeat the benzene extraction and centrifugation (steps 7 and 8) and add the second benzene extract to the wrapped centrifuge tube.

NOTE: The analysis of urine cannot be interrupted before the methylation step or recoveries of pentachlorothiophenol, tetrachloropyrocatechol, and tetrachlorohydroquinone will be low and erratic. This is true even with the addition of bisulfite.

VI. METHYLATION OF PHENOLS:

1. Concentrate the combined benzene extracts to a volume of 0.3-0.5 ml under a gentle stream of nitrogen in a 30°C water bath.

   NOTE: The extract is analyzed at this point, before derivatization, on a 5% DEGS column, which separates the two tetrachlorophenols (2,3,5,6 and 2,3,4,6) as the free phenols. These two phenols, when methylated, were not separated on any of the GC columns tested (Table 1).

2. Methylate the phenols with 5 ml of diazomethane reagent, prepared as described above in Subsection III,7.

3. Let the methylated extract stand for 1 hour.

4. Concentrate the solution to ca 0.3 ml under a gentle nitrogen stream.

5. Add 2 ml of hexane, and reconcentrate the solution to a volume of 0.2-0.3 ml.

VII. ACID ALUMINA COLUMN CHROMATOGRAPHY:

1. Preparation of columns:

   a. Loosely plug a size 22-9 Chromaflex column with a small amount of glass wool.

   b. Add 4.0 grams of acid alumina in small increments with tapping.

   c. Add 1.6 grams of anhydrous sodium sulfate on top of the alumina.

   d. Wash the adsorbents in the packed column free of interferences with 30 ml of hexane-benzene (60:40 v/v).

   e. Thoroughly air dry the column and place in an oven at 130°C overnight before use.

2. Cleanup and fractionation of methylated phenols:

   a. Remove a prepared column from the oven and let cool to room temperature.

   b. Add 7 ml of hexane to the column.

   c. When the solvent layer reaches the top of the sodium sulfate adsorbent, apply an aliquot of methylated sample or methylated standard phenol mixture in 0.2-0.3 ml to the column with a disposable pipet. To accomplish quantitative transfer of samples, rinse the centrifuge tube and pipet with three 0.5 ml volumes of hexane.

   d. Add an additional 3.5 ml of hexane, and collect and discard the total 5.0 ml hexane fraction.

   e. Elute the pentachlorophenol methyl ether with 20 ml of hexanebenzene (90:10 v/v). 2,3,4,6-Tetrachlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorothiophenol also elute in this Fraction I, if present in the extract.

   f. Elute 2,3,4,5-tetrachlorophenol, tetrachloropyrocatechol, and tetrachlorohydroquinone, if present, with 20 ml of hexane-benzene (60:40 v/v) (Fraction II).

   g. Adjust the fractions to an appropriate volume for EC GLC.

VIII. GAS CHROMATOGRAPHY:

Inject a portion, preferably 3-5 µl, of methylated PCP solution into the gas chromatograph operated with the following parameters:

Column	borosilicate glass, 1.8 m x 4 mm i.d.
Liquid phase	5% OV-210 coated on 80-100 mesh Gas-Chrom Q
Column Temperature	160°C
Carrier gas	argon-methane (95:5 v/v) flowing at 40 ml/minute
Detector temperature	300°C
Inlet	235°C
Transfer line	220°C
Detector	pulsed mode EC, 5 x 10^{-11} amp full scale

Under these conditions, 10 pg of PCP methyl ether gave a half-scale deflection with a retention of 0.49 relative to aldrin. Retention data for the seven phenol methyl ethers on 5% OV-210 and four other GLC columns useful for confirmation purposes are given below in Table 1. (See the Note in Subsection VI under item 1.)

TABLE 1. RELATIVE RETENTION DATA FOR METHYLATED METABOLITES OF HCB AND PCP

Retention time relative to Aldrin

Metabolite	4% Se-30-6% OV-210	1.5% OV-17-1.95% QFI	5% OV-210	3% OV-1	5% DEGS*
2,3,4,6-Tetrachlorophenol	0.23	0.33	0.24	0.22	1.21
2,3,5,6-Tetrachlorophenol	0.23	0.33	0.24	0.22	1.13
2,3,4,5-Tetrachlorophenol	0.38	0.51	0.46	0.34	1.66
Pentachlorophenol	0.46	0.55	0.49	0.44	2.58
Tetrachloropyrocatechol	0.45	0.55	0.52	0.42	--
Tetrachlorohydroquinone	0.48	0.56	0.59	0.42	--
Pentachlorothiophenol	0.95	1.06	1.00	0.91	--

* Undervitalized

Figures 1 and 2 illustrate the GLC separation on a 5% OV-210 column of the methyl ethers of the seven phenols after separation on the acid alumina column.

IX. DETECTION AND RECOVERY DATA:

1. Recoveries of PCP from urine at fortification levels of 5 ppb and greater averaged 90% when corrected for background PCP (Table 2).

TABLE 2. RECOVERY OF PCP FROM URINE[a]

ppm added	% range	av.% recov.	% rel.SD[b]
1.0	95.2-97.8	96.5	±1.1
0.3	92.3-99.0	95.3	±2.8
0.1	93.0-95.0	94.1	±0.8
0.003	91.9-100.4	95.1	±3.7
0.01	90.6-96.3	93.2	±2.5
0.005	88.0-104.0	93.9	±7.1

[a] Four determinations. [b] SD, standard deviation.

2. Recoveries of phenol metabolites from urine fortified at 10 ppb-1 ppm are listed in Table 3. Recoveries below 80% were obtained only for the two lowest concentrations of tetrachloropyrocatechol, the lowest concentration of tetrachlorohydroquinone, and all levels of pentachlorothiophenol. Addition of bisulfite and prompt execution of isolation procedures provide the maximum recoveries of these compounds.

TABLE 3. RECOVERIES OF METABOLITES FROM FORTIFIED URINE

Four determinations for each measurement.

Metabolite	ppm Added	Range (%)	Average Recovery (%)	Relative Stand. Deviation (%)
2,3,5,6-Tetrachlorophenol	1.0	89.3-92.3	91.1	±1.3
	0.3	85.7-92.3	88.8	±2.7
	0.1	82.0-87.9	85.3	±2.5
	0.03	78.0-85.6	82.3	±3.3
	0.01	79.1-87.5	82.8	±3.6
2,3,4,6-Tetrachlorophenol	1.0	88.9-92.4	90.9	±1.5
	0.3	86.1-91.8	88.9	±2.4
	0.1	83.1-88.3	86.0	±2.5
	0.03	80.8-84.2	82.5	±1.7
	0.01	79.6-86.8	82.6	±3.2
2,3,4,5-Tetrachlorophenol	1.0	89.3-95.6	93.1	±2.7
	0.3	89.0-94.3	91.8	±2.3
	0.1	86.0-91.0	88.2	±2.1
	0.03	85.8-90.3	87.4	±2.0
	0.01	82.8-90.5	85.6	±3.4
Pentachlorophenol	1.0	95.2-97.8	96.5	±1.1
	0.3	91.5-95.2	93.4	±1.8
	0.1	86.0-9.50	92.0	±4.1
	0.03	93.8-100.4	97.2	±2.8
	0.01	90.6-96.3	93.2	±2.5
Tetrachloropyrocatechol	1.0	78.6-81.4	80.1	±1.2
	0.3	78.7-85.7	81.6	±2.9
	0.1	76.3-83.0	79.8	±3.2
	0.03	59.1-71.4	65.6	±5.4
	0.01	60.1-69.7	63.7	±4.3
Tetrachlorohydroquinone	1.0	80.2-82.7	81.5	±1.0
	0.3	77.0-84.5	81.4	±3.2
	0.1	77.0-84.0	80.9	±2.9
	0.03	75.6-86.0	80.4	±4.6
	0.01	71.3-77.3	74.6	±2.5
Pentachlorothiophenol	1.0	69.9-73.6	71.9	±1.5
	0.3	69.3-73.3	71.1	±1.7
	0.1	61.0-73.0	66.4	±6.0
	0.03	49.8-57.2	53.3	±3.3
	0.01	41.5-51.3	47.3	±4.2

3. Method sensitivity was estimated to be 1 ppb for PCP and the other phenols in urine. Column cleanup was essential for determinations of PCP at levels below 30 ppb. When methods without cleanup were tested, recoveries of less than 80% were noted at fortification levels of 30 ppb or less.

4. The major metabolites from an HCB rat feeding study were tetrachlorohydroquinone, pentachlorothiophenol, and PCP. Minor rat urinary metabolites were 2,3,5,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol, and tetrachloropyrocatechol. Underivatized 2,3,5,6-tetrachlorophenol was separated from 2,3,4,6-tetrachlorophenol on a 5% DEGS column. The major metabolite isolated from a PCP rat feeding study was tetrachlorohydroquinone. Minor metabolites identified were 2,3,4,6-tetrachlorophenol and tetrachloropyrocatechol.

5. PCP was identified in ten of the eleven urine samples from the human general population, using the described analytical method. Levels ranged from 1 to 80 ppb (Table 4). The presence of 2,3,4,6-tetrachlorophenol in the urine can be attributed to its presence as an impurity in preparations of PCP. The only measurable metabolites from the general population samples were tetrachlorohydroquinone and tetrachloropyrocatechol. The occupationally exposed worker contained a high level of PCP and measurable levels of tetrachlorohydroquinone and tetrachloropyrocatechol. As can be seen from these results, pentachlorothiophenol in urine can be used as an indicator of possible exposure to HCB. PCP exposure would be indicated by a high level of PCP and the presence of tetrachlorohydroquinone and tetrachloropyrocatechol in the urine.

TABLE 4. HUMAN URINE

Results in ppm.

Sample No.	Penta-chloro-phenol	Tetra-chloro-hydro quinone	Penta-chloro-thiophenol	Tetra-chloropyro-catechol	2,3,4,6-Tetra-chlorophenol	2,3,5,6-Tetra-chlorophenol	2,3,4,5-Tetra-chlorophenol
1	0.006	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
2	0.012	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
3	0.004	<0.001	<0.001	0.002	0.003	<0.001	<0.001
4	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	0.080	0.002	<0.001	0.001	0.013	<0.001	<0.001
6	0.004	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
7	0.015	0.003	<0.001	0.004	0.004	<0.001	<0.001
8	0.012	0.006	<0.001	0.005	0.002	<0.001	<0.001
9	0.009	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
10	0.038	0.008	<0.001	0.007	0.009	<0.001	<0.001
11	0.018	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
12*	3.60	0.024	<0.001	0.024	0.123	<0.001	0.005

* Occupationally exposed to PCP.

X. CONFIRMATION BY CG-MS:

1. Confirm analytical results on a Finnigan Model 3200 quadrupole mass spectrometer equipped with a Model 9500 gas chromatograph and Model 6100 data system, or equivalent, with the following parameters:

Reagent	methane
Mode	chemical ionization
Source temperature	120°C
Pressure	900 μm
Electron energy	110 eV
Emission current	10 mA
GC column	borosilicate glass, 1.2 m x 2 mm i.d.
Liquid phase	5% OV-210 on 80-100 mesh Gas-Chrom Q
Column temperature	90°C isothermal for 1 minute, then programmed at 4°C per minute to 160°C
Carrier gas	20 ml/minute (methane)
Inlet temperature	200°C
Transfer line	250°C
Ion source	120°C

2. Chemical ionization using methane reagent gas produced fairly strong M + 1 quasi-molecular ion isotope clusters, beginning at m/e 245 for the three isomers of tetrachlorophenol, m/e 279 for PCP, m/c 295 for pentachlorothiophenol, and m/e 275 for tetrachloropyrocathechol and tetrachlorohydroquinone. In addition, a fiarly strong M + 1 quasi-molecular ion isotope cluster beginning at m/e 240 was tentatively identified as as an isomer of trichlorodihyroxybenzene from the PCP feeding study samples. The phenolic metabolites in the urine from the occupationally exposed worker were confirmed by GC-MS as tetrachloropyrocatechol and tetrachlorohydroquinone.

XI. CONFIRMATION OF PCP BY p-VALUE:

Equilibrate each of the following solvents, acetonitrile, methanol, and dimethylformamide, at a 1:1 v/v ratio with hexane at room temperature for 24 hours. Pipet 0.1 ml of a 0.5 ml hexane solution containing the PCP-methyl ether into a 1 ml test tube. Add, by means of a pipet, 0.1 ml of the hexane-equilibrated solvent and thoroughly mix the two phases by means of a Vortex mixer for approximately one minute. After the two phases separate, the upper hexane layer is ready for GLC analysis. The p-value is calculated as the concentration of PCP-methyl ether in the hexane phase divided by the concentration that was determined to be in the hexane before the partition. Determination of p-value from standards in each laboratory must be carried out at the same time as the unknown, because temperature and other variables affect the partition coefficients.

The expected p-values for the three solvent systems are:

1.	Acetonitrile:hexane	0.62
2.	Methanol:hexane	0.61
3.	Dimethylformamide:hexane	0.44

XII. MISCELLANEOUS NOTES:

1. Great difficulty was encountered in finding a control urine low enough in PCP content to use for fortification purposes. A general population human urine with an average 4 ppb PCP background was chosen for fortification purposes.

2. A comparison of PCP levels found in human urine samples by the method described in this section with two other procedures (including the one in this section in the last revision of this Methods Manual) indicated as much as a 17-fold higher result after hydrolysis.

3. Recoveries of 0.1-5 µg PCP and six phenolic metabolites of either HCB or PCP through the acid alumina column ranged from 88 to 97%.

XIII. ANALYTICAL QUALITY CONTROL:

1. All reagents, including water, must be extracted with hexane before use as they may be contaminated with PCP or other materials that may interfere with analysis. Glassware should be washed with dilute sodium hydroxide solution, followed by deionized water and acetone rinses. Care should be taken not to allow contact between wooden or paper materials and glassware because peg boards and several brands of absorbent paper products have been found to contain PCP.

2. Fortified urine samples should be analyzed along with each series of actual samples to verify adequate recovery of PCP and the other phenols of interest. Because of the ubiquity of PCP, the "blank" used for fortification must be analyzed and a correction must be made for the amount of PCP found.

3. A reagent blank consisting of 5 ml of pre-extracted distilled water should also be carried through the entire procedure along with the sample(s).

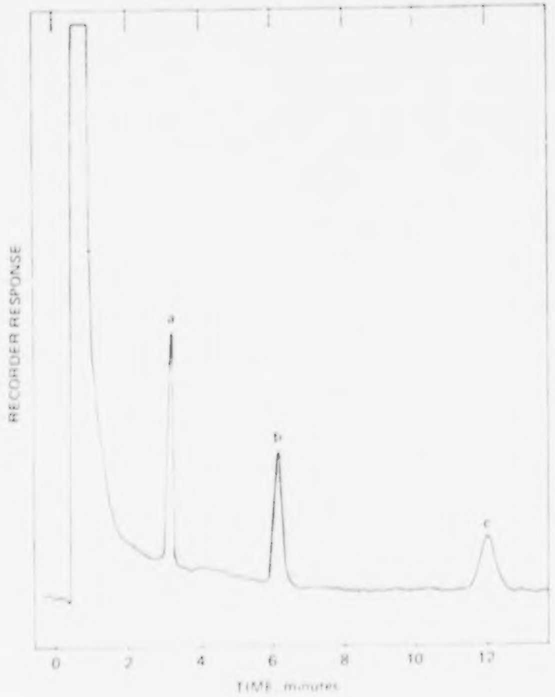

Fig. 1. Gas chromatogram of Fraction I from acid alumina column of standard phenol methyl ether mixture: (a) 2,3,5,6- and 2,3,4,6-tetrachlorophenol (See Note, Section VI, 1); (b) pentachlorophenol; (c) pentachlorothiophenol. Column, 5% OV-210 on 80-100 mesh Gas-Chrom Q. Oven temperature, 160°C. 5% Methane in argon, flow rate 40 ml/minute.

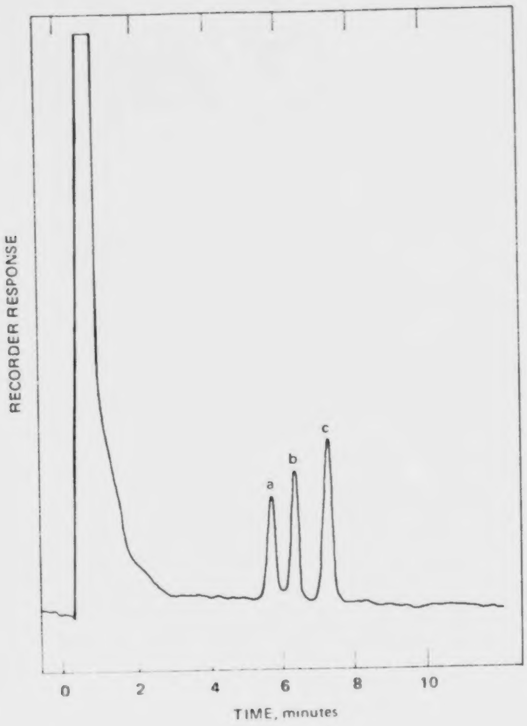

Fig. 2. Gas chromatograms of Fraction II from acid alumina column of standard phenol methyl ether mixture: (a) 2,3,4,5-tetrachlorophenol; (b) tetrachlorocatechol; (c) tetrachlorohydroquinone. Column, 5% OV-210 on 80-100 mesh Gas-Chrom Q. Oven temperature, 160°C. 5% Methane in argon, flow rate 40 ml/minute.

DETERMINATION OF BIS(p-CHLOROPHENYL) ACETIC ACID (DDA) IN HUMAN URINE

I. INTRODUCTION:

The analysis of blood, urine, and feces is of extreme importance when studying transport and elimination of p,p'-DDT and p,p'-DDT - derived metabolites. The examination of urine is of particular interest because of the ease of collection and the anticipation of fewer analytical problems than might be encountered with blood and feces. Furthermore, a predominant metabolite of p,p'-DDT, p,p'-DDA, is excreted in the urine. Excretion levels of this metabolite have been established as sensitive indicators of exposure to p,p'-DDT (Durham et al., 1965). However, a rapid, sensitive gas chromatographic procedure for the analysis of this metabolite is desirable, particularly one which gives accurate and precise data for low levels of p,p'-DDA excretion. The following method was developed as a dual analytical procedure to determine DDT and its polar and non-polar metabolites in human urine (Cranmer et al., 1969). Utilizing electrolytic conductivity or microcoulometric detection, the procedure can be readily adapted for the exclusive determination of p,p'-DDA excretion levels.

REFERENCES: Cranmer, M. F., J. J. Carrol, and M. F. Copeland (1969) Determination of DDT and Metabolites Including DDA in Human Urine by Gas Chromatography. Bull. Environ. Contamin. & Toxicol. 4, 214.

Cueto, C., A. G. Barnes, and A. M. Mattson, (1956). Determination of DDA in Urine Using an Ion Exchange Resin. J. Agr. Food Chem. 4, 943.

Durham, W. F., J. F. Armstrong, and G. E. Quinby (1965). DDA Excretion Levels, Arch. Environ. Health 11, 76.

II. PRINCIPLES:

Each sample of urine is thoroughly mixed with an equal volume of 2% acetic acid in hexane. Three such extractions are performed and the combined extracts evaporated to near dryness taking care that no residual traces of water or acetic acid remain. The dry extract is treated with boron trifluoride-methanol reagent to convert free p,p'-DDA to the methyl ester. After heating at 50°C for 30 minutes, the reaction is quenched with water and the reaction mixture is then extracted with three 5-ml portions of hexane. The combined hexane extracts are volume adjusted and the *p*,*p*'-DDA methyl ester is determined by microcoulometric and/or EC detection. An osmolality correction factor is employed in reporting *p*,*p*'-DDA excretion levels.

III. EQUIPMENT:

1. Gas chromatograph equipped with EC detector and microcoulometer if available, and with the columns prescribed for the program.
2. Vortex-Genie mixer, Model 55Q-G or the equivalent.
3. Precision Systems Osmette, Model 20Q7 or the equivalent.
4. Culture tubes, screw caps with Teflon liners, 16 x 125 mm, Corning No. 9826.
5. Culture tubes, screw caps with Teflon liners, 20 x 150 mm, Corning No. 9826.
6. Separatory funnels, Teflon stopcocks, 60 ml, 125 ml and 250 ml.
7. Concentrator tubes, 10 ml and 25 ml capacity, graduated with Ŧ 19/22 ground glass joint (Kontes Glass Co., Cat. No. K-570050, size 1025 (10 ml) and size 2525 (25 ml).
8. Modified microSnyder column, Ŧ joint size 19/22, Kontes No. K-569251.
9. Kuderna-Danish flasks, 125 ml and 250 ml, Kontes No. K-570001.
10. Glass beads, solid, 3 mm.
11. Water bath(s), controllable at temperatures of 45°, 60°and 95°to 100°C.
12. Mohr pipets, 5- and 10-ml.
13. Disposable pipets.
14. Filter tubes, 150 x 24 mm, Corning No. 9480 or the equivalent.
15. Micro Florisil column per specifications given in Section 5,A,(2),(a), page 1.
16. Test tubes, 25 x 200, with Ŧ glass stoppers, Corning No. 9810 or the equivalent.

IV. REAGENTS:

1. Acetic acid, glacial, reag. grade.
2. Hexane
3. Acetonitrile — All four solvents of pesticide quality.
4. Toluene
5. Methanol
6. Sodium sulfate granular, anhydrous, reag. grade.
7. A mixture of 2% acetic acid in hexane.
8. A solution of 1% methanol in hexane.
9. Boron trifluoride, reag. grade, lecture bottle size, The Matheson Company, East Rutherford, N. J.
10. p,p'-DDA analyt. standard, available from EPA, Reference Standards Repository, Research Triangle Park, N. C.
11. Preparation of esterification reagent:

    Bubble boron trifluoride rapidly into cool methanol for 1 hour, stirring continuously by mechanical stirrer and passing a slow stream of dry nitrogen over the surface of the methanol to continuously purge the reaction flask. A weight increase of a ca 10% should be observed during the course of preparation of the reagent. Consistent results should be obtained with reagent which is stored in tightly capped bottles in the refrigerator for periods up to 2 weeks.

    NOTE: An alternative to the preparation of the methylating reagent is to purchase the commercially prepared reagent. Applied Science Laboratories markets a "BF_3 METHANOL ESTER KIT" consisting of 25 x 5 ml ampoules of 14% BF_3/Methanol. (Nanograde methanol will be substituted on request.)

12. Preparation of DDA-ME standard:

    1. Weigh out approximately 25 mg of Bis- (p-chlorophenyl)-acetic acid (p,p'-DDA) into 25 x 200 mm glass-stoppered round bottom test tube. (Item 16, Subsection III)

2. Dissolve the p,p'-DDA, with the aid of a Vortex mixer, in 10.0 ml of BCl_3-Methanol 10% w/v.

3. Place in steam bath for 30 minutes.

4. Remove from steam bath and quench reaction by addition of 10 ml ice-cold distilled water.

5. Extract three times with 10 ml portions of hexane, filtering each extract through sodium sulfate into previously tared concentrator tubes.

6. Concentrate to small volume after each hexane extract.

7. After last hexane extract, rinse down sidewalls of the concentrator tube with a small amount of hexane and then evaporate just to dryness under gentle stream of nitrogen at room temperature.

8. Place concentrator tube in desiccator and allow to equilibrate.

9. Reweigh the concentrator tube to determine the amount of DDA-methyl ester.

$$\%\ \text{Recovery} = \frac{\text{mg of DDA(ME)} \times 280}{\text{Mg of DDA} \times 294} \times 100$$

10. The DDA-methyl ester is then quantitatively transferred to 50 ml volumetric flask with nanograde hexane to prepare the DDA-methyl ester standard of approximately 1 mg/ml.

11. This DDA-methyl ester standard may then be further diluted to give working standard of the desired concentration.

V. SAMPLE COLLECTION AND PREPARATION:

Urine collections are made in scrupulously cleaned, screw-cap (Teflon or foil-lined) bottles to which 1 ml of toluene has been added as a preservative. Donors may be requested to collect their specimens immediately after arising in the morning. Pooled, 24 hour urine specimens may be desirable in those cases where samples are suspected or known to have p,p'-DDA concentrations approaching the lower limit of detectability. The volume of urine extracted will vary depending on exposure classifications. For analysis of the urine of individuals classified as "normal", 20-50 ml should be available.

In those cases where known or suspected exposure to p,p'-DDT has occurred, 5-10 ml of urine may be sufficient. The osmolality of each specimen is determined shortly after receipt using a Precision Systems Osmette. Samples to be stored prior to analysis should be kept in a refrigerator.

VI. EXTRACTION:

A control sample of urine from an unexposed donor should be carried through the entire procedure parallel with the sample(s) being tested.

1. Place the urine sample in the extraction vessel of appropriate type and size for the volume of sample and add an equal volume of 2% acetic acid in hexane.

   NOTE: A 5 ml sample can be extracted in a 16 x 125 mm culture tube with Teflon lined screw cap. A 10 ml sample will require the 20 x 150 mm tube. Volumes of 15 to 20 ml and 25 to 50 ml may be extracted in sep. funnels of 60 and 125 ml, respectively.

2. Shake vigorously for 2 minutes using hand agitation for the sep. funnels or the Vortex mixer for culture tubes.

   NOTE: Some emulsion may result from the vigorous shaking. The test tubes may be centrifuged to break the emulsion. If emulsions persist in sep. funnel or tubes, add a few drops of acetonitrile.

3. The extraction is repeated twice more to insure complete extraction of pesticides into the solvent phase. The method of conveniently handling the repetitive extractions will depend upon the initial volume of sample and subsequent total volume of the three combined extracts. The following options are based on this volume factor:

   a. For 5 ml urine samples the 15 ml of combined hexane extract is collected in a 25 ml grad. evap. concentrator tube containing one 3 mm glass bead. The extract transfer is made with a 5 ml Mohr pipet. The conc. tube is fitted with a modified microSnyder column and the extract is concentrated in a boiling water bath to ca 2 ml.

   b. A urine sample of 10 ml will result in a total combined extract volume of 30 ml. In this case, transfer each 10 ml extract into a 50 ml grad. beaker by means of a 10 ml Mohr pipet. On a 45°C bath, evaporate the solvent under a nitrogen stream to ca 5 ml. Cool beaker, add a pinch of anhydrous Na_2SO_4 and transfer concentrate to a 25 ml evap. concentrator tube, rinsing beaker with three portions of 4 ml each of hexane. Proceed with concentration as outlined in step a, above.

c. For initial urine samples of 15 to 25 ml which are extracted in sep. funnels, evaporation in Kuderna-Danish equipment is suggested. Draw off the aqueous (lower) layer from the first extraction into a second sep. funnel and filter the hexane extract through a filter tube containing a 2-in. column of anhydrous Na_2SO_4 into a 125 ml K-D flask fitted with a 10 ml grad. evap. concentrator tube containing one 3 mm glass bead. Add a like volume of the acetic acid/hexane reagent to the urine phase in the second sep. funnel, stopper, and shake vigorously 2 minutes. After layer separation, draw off the aqueous layer into sep. funnel No. 1 and the hexane extract through the Na_2SO_4 filter into the K-D flask. Similarly, repeat the extraction a third time, conducting the extraction in sep. funnel No. 2. Attach a Snyder column to the K-D flask, place lower conc. tube in a boiling water bath and reduce extract to ca 2 ml.

d. For initial urine samples of 30 to 60 ml, the extraction is conducted identically to that outlined in step c above except that a 250 ml K-D flask is used to accommodate the larger volume of combined extract.

4. The 25 ml evap. concentrator tubes will require two successive rinses of ca 3 ml each with hexane to insure removal of any residue-containing material adhering to the sides of the tube or around the joint. After each 3 ml rinse, applied by disposable pipet, the extract is further concentrated down to ca 2 ml. The self flushing action of the K-D assemblies should take care of this problem except for a wash of the joint between K-D flask and evap. concentrator tube. The final concentrated extract should be ca 2 ml.

5. The final concentrated extract of ca 2 ml will be in a 10 or 25 ml evap. concentrator tube. This is placed in a 45° water bath and reduced just to dryness under a dry nitrogen stream.

VII. ESTERIFICATION:

1. Add 2.5 ml of the methylation reagent to the dry extract in the evap. concentrator tube. Place tube in a 50°C bath and hold for 30 minutes.

   NOTE: If the 14% commercial methylating reagent is used, the volume of reagent may be reduced to 2.0 ml.

2. Quench reaction by adding 5 ml of dist. H_2O.

3. Add 5 ml hexane, stopper tube, and mix on Vortex 1 minute. Allow layers to separate.

4. With a 5 ml Mohr pipet, transfer the hexane layer to a clean 25 ml evap. concentrator tube containing one 3 mm glass bead.

5. Repeat the extraction twice more with like volumes of hexane, combining the three 5 ml extracts in the 25 ml evap. concentrator tube.

6. Attach a modified micro-Snyder column and reduce the volume of the extract to ca 3 ml in a boiling water bath.

7. Remove tube from bath, cool, rinse joint with a small volume of hexane applied with a disposable pipet, place tube under a dry nitrogen stream and reduce extract volume to 0.3 ml.

VIII. FLORISIL FRACTIONATION:

1. Micro Florisil columns are prepared ahead of time and held in a 130°C oven until ready for use. Detailed instructions for preparing the column are given in Section 5,A,(2),(a), page 1.

2. Remove micro column from oven and allow to cool to room temperature, then prewet column with 10 ml of hexane, discarding eluate.

3. Proceed with the elution as described in Section 5,A,(2),(a), Subsection V, Steps 3 through 8.

   NOTES:

   1. After it has been established by trial that all of the DDA is eluted in the second fraction and none in the first fraction, the eluate from the first fraction can be discarded if DDA is the sole compound of interest.

2. If a laboratory is running routine determinations as for surveillance of a special group of donors, and is conducting gas chromatography by microcoulometric detection only, the Florisil cleanup step may be eliminated. However, the cleanup is necessary when the detection technique is electron capture.

IX. GAS CHROMATOGRAPHY:

1. Urine from general population donors may be expected to yield as little as 8 ppb of DDA. A 50 ml initial sample concentrated to a final extract volume of 300 μl would yield an approximate DDA concentration of 1.3 nanograms per microliter. A 10 μl injection should produce a quantifiable peak via EC under normal conditions. An exploratory injection of 25 μl for MC detection will provide the operator with information suggesting a lesser or greater injection volume to obtain a peak height response of 10% or more FSD.

2. Compare the peak heights of the sample p,p'-DDA methyl ester with the peak heights produced by injection of a standard solution of p,p'-DDA methyl ester of known concentration. Correct the observed concentration levels of p,p'-DDA in the urine samples to an osmolality of 1000 milliosmols by multiplying the calculated value by a correction factor, K, given by the following expression:

$$K = \frac{1000}{\text{Observed Osmolality}}$$

3. The only potential pesticide interference to the DDA, methyl ester, peak in the second fraction eluate would be from dieldrin on the OV-17/QF-1 column operated at the prescribed 200°C. The SE-30/QF-1 and the OV-210 columns, when operated at their prescribed parameters, should offer no overlap problems. The OV-210 column is particularly recommended because of its greater responsiveness.

   The urine from high exposure donors would be expected to contain a small amount of p,p'-DDE as compared to the DDA levels. When the OV-17/QF-1 and SE-30/QF-1 columns are operated at the prescribed 200°C temperature, the peaks would overlap. Complete separation can be obtained however, by operating at 170°C. The OV-210, operated at its prescribed temp. of 175-180° should provide complete separation of the compounds.

DETERMINATION OF 2,4-D AND 2,4,5-T IN URINE

I. INTRODUCTION:

A number of derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are applied extensively as selective herbicides in the control of terrestrial and aquatic broadleaf plants. Because of their widespread use and relatively lengthy persistence, particularly in treated lakes and streams, potential human exposure to these materials may occur via several routes. These include consumption of contaminated edible plants, livestock, and water, as well as direct exposure by agricultural spraymen and herbicide formulators. Thus, rapid, sensitive procedures for the detection of the free acids and chlorinated phenol degradation products in human and animal urine assumes an important role in the toxicological and environmental monitoring of these herbicidal compounds.

REFERENCE: A Method for Determination of Low Levels of Exposure to 2,4-D and 2,4,5-T, Shafik, M. T., Sullivan, H. C. and Enos, H. F., Journal of Environmental Analytical Chemistry, 1971, Vol. 1 pp 23-33.

II. PRINCIPLE:

The phenolic conjugates are subjected to acid hydrolysis, the free phenols and acids are extracted and ethylated with diazoethane. Cleanup of the derivatized products is carried out on a silica gel column, the resulting eluate is concentrated to an appropriate extent and subjected to analysis by electron capture GLC, chromatographing on a column of 4% SE-30/6% OV-210.

III. EQUIPMENT:

1. Gas chromatograph with EC detector fitted with a glass column 6 ft. x 1/4 in. o.d. packed with 4% SE-30/6% OV-210. Column and instrumental parameters are those prescribed in Section 4A. Injection port, transfer line and detector as maintained in normal operation.
2. Chromatographic columns, Size 22, Kontes No. 420100.
3. Boiling water or steam bath.

4. Distilling column (condenser), 200 mm jacket, fitted with tight glass stopper at top, Kontes No. 286810.

5. Circulating water pump.

6. Vortex mini-mixer.

7. Evaporative concentrator tubes, grad., 25 ml \$ 19/22, Kontes No. 570050.

8. Conical centrifuge tubes, conical, grad., 15 ml with \$ stoppers, Corning No. 8084 or the equivalent.

9. Disposable pipets, Pasteur, 9-in.

10. Dry nitrogen. Tank fitted with 2-stage pressure regulator.

11. Volumetric flasks, 50 and 100 ml.

12. Mohr pipets, 0.2, 0.5 and 5 ml.

13. Transfer (vol) pipets, 1 through 5 ml.

14. An exhaust hood with a minimum draft of 150 linear feet per minute.

15. Centrifuge capable of 2,000 rpm.

IV. REAGENTS:

1. Benzene, pesticide quality.

2. Hexane, pesticide quality.

3. Hydrochloric Acid, conc., A.R. grade.

4. Silica gel, Woelm, activity grade I.

   NOTE: Dry adsorbent for 48 hours at 170°C and store in a desiccator. On day of use, deactivate the silica gel by adding 15 µl of water and 1 gram of silica gel to a 125 ml Erlenmeyer flask. Stopper and rotate until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hours with periodic shaking. Prepare the chromatographic columns just prior to use.

5. N-ethyl-N'-nitro-N-nitrosoquandine, Aldrich Chemical Co.

6. Distilled water. All distilled water used throughout procedure must be benzene extracted.

7. Ethylating Reagent, Preparation:

   a. In a 125 ml Erlenmeyer flask, dissolve 2.3 grams of KOH, A.R. grade in 2.3 ml of distilled water. When solution is complete, allow to cool to room temperature.

   b. Add 25 ml hexane and cool flask in a -18°C freezer for 15 min.

   c. In a VERY HIGH DRAFT hood, add 1.6 grams of N-ethyl-N'-nitro-N-nitrosoguanidine in small portions at a time, mixing contents of flask after each addition.

   d. Decant the hexane layer into a bottle with a Teflon-lined screw cap. This may be stored for periods up to a week at -18°C.

   NOTES:

   1. Because of demonstrated carcinogenicity and toxicity, do not allow the nitrosoguanidine of the diazoethane to come in contact with the skin. Disposable gloves and safety goggles should always be worn when handling.

   2. Do not use ground glass stoppered bottles or bottles with visible interior etching.

8. Analytical grade standards for 2,4-D and 2,4,5-T. Available from the EPA Reference standards Repository at Research Triangle Park, NC.

9. Preparation of ethylated standard mixtures;

   a. Weigh 20 mg of each of the two analytical standards into separate 100 ml vol. flasks, dissolve, and make to volume with benzene. These concentrated stock solutions will contain 200 ng/µl each of the two compounds.

   b. Transfer aliquots from each of the concentrated stock solutions into a single 50 ml vol. flask in the following volumes:

      2,4-D -------- 1.0 ml   2,4,5-T -------- 0.5 ml

c. Add diazoethane dropwise with a disposable pipet until a definite yellow color persists.

d. Allow solution to stand 15 minutes, then bubble nitrogen through the solution until yellow color disappears (ca 5-10 minutes). THIS OPERATION MUST BE DONE IN A HIGH DRAFT HOOD. Dilute to volume with benzene. This is the alkylated stock standard mixture of the following concentrations:

2,4-D-------4 ng/µl   2,4,5-T-------2 ng/µl

e. Prepare an ethylated working standard mixture of highest usable concentration by pipetting 5 ml of the alkylated stock mixture (d. above) into a 50 ml vol. flask and make to volume with benzene. This will yield a dilute mixture of the following concentrations:

Alkylated 2,4-D---------------------400 pg/µl
Alkylated 2,4,5-T-------------------200 pg/µl

Injection of 5 µl of this mix into the gas chromatograph will provide information on the final concentration range needed for further diluted standards.

NOTE: These alkylated standards should be stored at -18°C when not in use and discarded after one month.

V. EXTRACTION AND ALKYLATION:

A control sample of urine from an unexposed donor should be carried through the entire procedure parallel with the sample(s) being tested.

1. Pipet 1 to 5 ml of urine into a 25 ml evap. conc. tube.

   NOTE: The precise volume is predicated on the expected residue level.

2. Add dropwise a volume of conc. HCl equal to 1/5 the volume of urine, and mix well.

3. Fit a stoppered reflux condenser to the tube and heat in boiling water bath for 1 hour, cooling the condenser with circulating ice water.

4. Remove from bath, cool, and rinse inside walls and condenser tip with 3 ml benzene.

5. Mix contents of tube for 2 minutes on a Vortex set at high speed and then centrifuge at 2,000 rpm.

6. By means of a disposable pipet, carefully transfer the benzene (upper) layer to a 15 ml centrifuge tube taking special care not to transfer any water.

7. Repeat the extraction with another 3 ml portion of benzene, adding the second benzene to the centrifuge tube.

8. Add diazoethane reagent dropwise with a disposable pipet until the yellow color persists (ca 2 ml).

9. Allow tube to stand 15 minutes, then bubble nitrogen through the solution to remove excess reagent.

10. Concentrate the ethylated extract to ca 0.3 ml at room temperature or on a 40°C water bath under a gentle stream of nitrogen.

VI. SILICA GEL FRACTIONATION:

Determination of Elution Pattern

The elution pattern of the ethylated compounds must be determined before using the silica gel column for cleanup of the ethylated urine extracts. The column preparation and elution pattern evaluation is outlined in the following steps:

a. Place a small wad of glass wool at the bottom of a Chromaflex column and add 1 gram of the particularly deactivated silica gel. Top this with 1/2 in. of anhydrous, granular Na_2SO_4.

b. Prewash the column with 10 ml of hexane and discard the eluate.

c. When the surface level of the hexane reaches a point on the column ca 2 cm from the top of the Na_2SO_4, add 0.3 ml of the alkylated stock standard mixture (Subsection IV,9,c) to the column. Elute successively with 10 ml of each of the solvent systems listed in the following table, collecting each fraction separately. Inject from 5 to 10 μl from each fraction into the gas chromatograph and calculate the percent of each compound present in the fraction.

A typical elution pattern is shown in the table:

Eluting Solvents	2,4-D	2,4,5-T
	5 μg	2 μg
20% Benzene-Hexane	0	0
40% Benzene-Hexane	0	0
60% Benzene-Hexane	0-2%	20-25%
80% Benzene-Hexane	98-100%	75-80%
100% Benzene	0	0

Sample Fractionation

a. Prepare a chromatographic column of silica gel as described on the previous page and prewash column with 10 ml of hexane, exactly as described, discarding the elute.

b. Transfer the concentrated extract to the column, rinsing centrifuge tube with two successive portions of 5 ml each of 20% benzene/hexane, collecting the elute.

NOTE: If chlorinated phenols are present they should elute in this fraction.

c. Finally, add 10 ml of 60% benzene/hexane followed by 10 ml of 80% benzene/hexane, collecting both these fractions in a single tube. The ethylated 2,4-D and 2,4,5-T are contained in these fractions.

NOTE: If the individual analyst has determined that his elution pattern differs from that given in the author's table and he is able to obtain a consistent altered pattern, some appropriate revision in the eluate collection instructions may be indicated.

VII. GAS GHROMATOGRAPHY:

Inject into the gas chromatograph 5 to 10 μl of the 20% fraction for the determination of the phenols and 5 to 10 μl of the combined 60-80% fraction for the determination of the chlorophenoxyacetic acids. Injections of 5 to 10 μl can also be made from fractions which have been concentrated to 5 ml, if necessary, in case of lower levels of exposure. The elution pattern of the 2 compounds extracted from a fortified urine sample must be established as described below. The limits of detectability for 2,4-D and 2,4,5-T are 0.05 and 0.01 ppm, respectively. Quantification is conducted by mathematical comparison of sample peaks against peaks resulting from the injection of working standard (Subsection IV,9,e).

The retention values, relative to aldrin, of the two ethylated compounds on the SE-30/QF-1 column at 200°C are:

2,4-D-------0.51	2,4,5-T-------0.78

VIII. MISCELLANEOUS NOTES:

1. Recovery runs are essential for the operator to determine the efficiency of alkylation and cleanup. From the concentrated stock standard of IV,9,a, transfer the aliquots specified in Step 9,b to a single 50 ml vol. flask. Dilute to volume with benzene without ethylating. Transfer a 2 ml aliquot to a 15 ml grad. centrifuge tube and add an equal volume of 1 N NaOH. Mix well and allow to stand for 10 minutes, agitating from time to time. Centrifuge 5 minutes at 2,000 rpm and discard benzene (upper) layer. Fortify 5 ml of control urine with aliquots of 0.1 to 1 ml of the aqueous extract and proceed as described in Subsection V starting at Step 2.

2. Because of differences in ambient temperature and relative humidity from one laboratory to another, it is imperative that each laboratory establishes silica gel elution patterns under local conditions. Should the compounds of interest elute in a later fraction (i.e., in 100% benzene instead of 60% or 80% benzene-hexane) the percent water added to the silica gel must be increased by 1% increments until desired elution pattern is established. If the compounds of interest elute in an earlier fraction (i.e., in 20% B-H instead of 60% or 80% B-H), the amount of water initially added to silica gel must be decreased (use spiked control urine, not standard compounds to determine pattern).

DETERMINATION OF KEPONE IN HUMAN BLOOD AND ENVIRONMENTAL SAMPLES

I. INTRODUCTION:

Kepone (chlodecone) is a pesticide added to bait or other inert material to control banana and potato pests and to serve as a potent ant and roach killer. It is an ingredient in about 55 commercial pesticide formulations used in the United States and other countries. The following methods describe the analysis of Kepone in human blood, air, river water, bottom sediments, and fish as carried out by the EPA Health Effects Research Laboratory, Research Triangle Park, NC.

REFERENCES:

1. Electron Capture Gas Chromatographic Determination of Kepone in Environmental Samples, Moseman, R. F., Crist, H. L., Edgerton, T. R., and Ward, M. K., Arch. Environ. Contam. Toxicol. 6, 221 (1977).
2. A Micro Technique for Confirmation of Trace Quantities of Kepone, Moseman, R. F., Ward, M. K., Crist, H. L., and Zehr, R. D., J. Agr. Food Chem. 26, 965 (1978).
3. Analytical Methodology for the Determination of Kepone Residues in Fish, Shellfish, and Hi-Vol Air Filters, Hodgson, D. W., Kantor, E. J., and Mann, J. B., Arch. Environ. Contam. Toxicol. 7, 99 (1978).
4. Mass Spectrometric Analyses and Characterization of Kepone in Environmental and Human Samples, Harless, R. L., Harris, D. E., Sovocool, G. W., Zehr, R. D., Wilson, N. K, and Oswald, E. O., Biomed. Mass Spectrom. 5(3), 232 (1978).
5. Preliminary Report on Kepone Levels in Human Blool from the General Population of Hopewell, VA, U.S. EPA, HERL, Research Triangle Park, NC, March 3, 1976.

II. PRINCIPLE:

Samples are extracted, and extracts are cleaned up by chromatography on a micro Florisil column, base partitioning, or gel permeation chromatography. The Kepone is determined by electron capture gas chromatography with multiple columns. Confirmation of

Kepone in samples is possible by several procedures: (1) chemical derivatization that converts Kepone to mirex followed by further cleanup and analysis by EC GLC; (2) use of the halogen selective Hall conductivity GLC detector; or (3) chemical-ionization mass spectrometry (methane reagent gas) coupled with gas chromatography.

III. SAMPLE COLLECTION:

Samples of water, sediment, soil, ice, and sludge are collected in 1-quart (0.946 L) Mason jars previously washed and solvent treated according to procedure given in Section 3,A of this Manual. Jar lids should be lined with Teflon or aluminum foil. Fish samples are wrapped in foil and frozen along with other solid samples.

All samples should be refrigerated as soon as possible after collection and the refrigeration maintained until the start of the analysis.

The following methods are intended for the analysis of Kepone residues in human blood, air (sampled with Hi-Vol filters), water (and ice), sediment, soil, sludge, fish, and shellfish.

IV. APPARATUS AND REAGENTS:

See Section 5,A,(2),(a), III for materials and reagents for the micro Florisil cleanup method. Additional requirements follow:

1. Gas chromatograph fitted with a DC or pulsed linearized mode electron capture detector. GLC columns, boroscilicate glass, 1.8 m x 4 mm i.d., packed with 3% OV-1 or 1.5% OV-17/1.95%. OV-210 on 80-100 mesh silanized support, operated with specific parameters given under Gas Chromatography, Section XII. Criteria for high sensitivity in the GLC system, as set forth in Section 4,A,(4), page 4 for EC detection should be carefully noted. Alternative columns for confirmation are 4% SE-30/6% OV-210, 5% OV-210, and 5% OV-1.

   NOTE: It should be noted that on the 4% SE-30/6% OV-210 column, Kepone and p,p'-DDD co-elute. This may prove troublesome if the column promotes conversion of p,p'-DDT to p,p'-DDD.

2. Culture tubes, 125 x 15 mm and 77 x 15 mm, with Teflon-lined screw caps.

3. Chromatographic columns, Chromaflex 22-7, Kontes Glass Co.

4. Pipets, Pasteur disposable.

5. Mechanical rotator producing a tumbling action at ca 50 rpm.

6. Centrifuge tubes, conical, 15 ml, graduated.

7. Apparatus for evaporation of solutions held in a 60°C water bath under a gentle stream of purified nitrogen gas.

8. Pipet, Mohr, 10 ml, graduated in 0.1 ml increments.

9. Centrifuge tube, 50 ml, graduated, with screw cap.

10. Tube, round bottom, 50 ml, with screw cap.

11. Separatory funnels, 125 ml and 1000 ml, with Teflon stopcock.

12. Soxhlet extraction apparatus, size 50 x 250 mm.

13. Erlenmeyer flask, 125 ml, glass-stoppered.

14. Food chopper, Hobard, Model 84142.

15. Duall tissue grinder, number K-885450, Kontes Glass Co.

16. Polytron homogenizer, Brinkmann Instruments.

17. Volumetric flask, 100 ml capacity.

18. Waring Blender.

19. Sorvall Omni-Mixer, Type OM.

20. Vacutainer tube, holder, and needle blood collection system.

21. 3-Ball Snyder column.

22. Analytical pesticide standards, prepared from analytical grade Kepone, available for qualified laboratories from the Reference Standards Repository, ETD, HERL, EPA, Research Triangle Park, NC. Prepare stock solutions in pesticide quality benzene, and prepare the final working solution from an intermediate dilution with 1% methanol in benzene. The use of 1-2% methanol in benzene is mandatory for all standards and samples in order to obtain maximum electron capture GLC response.

23. Solvents, all of pesticide quality.

24. Sodium sulfate, reagent grade, Soxhlet extracted for 6 hours with pesticide quality benzene or methylene chloride and oven dried at 130°C before use.

V. ANALYSIS OF HUMAN BLOOD:

1. Collect blood samples in Vacutainer. Samples should contain no anti-clotting agent. Prepare a homogeneous sample by breaking up the clots with a flat-end glass rod of a diameter nearly as great as the inside diameter of the collection tube.

2. Using an open tipped Pasteur pipet, weigh 2.0 g of blood into a culture tube with a Teflon-lined screw cap.

NOTES:

1. Small blood clots tend to clog the tip of a normal disposable pipet. For this reason, break off the tip to provide a greater inside diameter.

2. From this point on, run spiked blood and reagent blanks through the entire procedure in exactly the same manner as the samples.

3. Add 6 ml of hexane-diethyl ether (1:1 v/v) to the tube and cap securely.

4. Shake the tube on a mechanical rotator at 30-50 rpm for 30 minutes.

5. Centrifuge the extraction mixture at 3000 rpm for ca. 5 minutes to eliminate emulsions.

6. Remove the solvent layer with a disposable pipet and transfer to a clean 15 ml graduated centrifuge tube.

7. Repeat the extraction with an additional 6 ml of the hexane-diethyl ether (1:1 v/v), combining the extracts in the 15 ml centrifuge tube.

8. Evaporate the solution in the centrifuge tube to 0.20 ml under a gentle stream of nitrogen.

9. Carry out Florisil column cleanup as follows:

   a. Prepare and activate a micro column containing 1.6 grams of 60-100 mesh Florisil (activated by the manufacturer at 1200°C) topped by 1.6 grams of anhydrous sodium sulfate as described in Section 5,A,(2),(a),III, 1 and 2.

NOTE: Prewash the column with 30 ml benzene-methanol (1:1 v/v) rather than with hexane followed by methanol as specified in Section 5,A,(2),(a). Let the column air dry thoroughly before overnight oven activation at 130°C.

b. Remove the prepared column from the oven and cool. Prewet the column with 10 ml of methanol-benzene-hexane (2:4:94 v/v) and discard the eluate.

c. When the solvent level reaches the top of the Na_2SO_4, transfer the total sample extract to the column with a disposable pipet, rinsing the tube with three 0.5 ml portions of the same methanol-benzene-hexane solvent and adding these to the column with the same pipet. Begin to collect the column effluent in a clean 15 ml centrifuge tube as soon as the addition of the sample is begun.

NOTE: The sample aliquot should not exceed 500 mg contained in a 0.2-0.3 ml volume.

d. Elute the column with an additional 5.5 ml of this solvent, added to the column using a 10 ml pipet. The first 7.0 ml collected to this point (Fraction I) is discarded. This fraction should contain the PCBs, mirex and several additional chlorinated pesticides, if present.

e. Add 30 ml of methanol-acetonitrile-benzene-hexane (1:2:4:93 v/v) and collect the effluent in a clean 50 ml centrifuge tube. This is Fraction II, which contains the Kepone. Dieldrin and endrin, if present in the sample, will be partially recovered in this fraction.

NOTE: Recovery of Kepone at very low levels (5-30 ng) through a Florisil column is only semiquantitative. At higher amounts, recovery is at least 90%.

Concentrate the solution under a stream of nitrogen to an appropriate volume for injection of a 5 μl sample into the EC GLC.

NOTES:

1. At a screening level of 1 ppb, a 2.0 g sample would contain 2.0 ng (assuming 100% recovery), so the injected aliquot must contain a fraction of this final solution consistent with the sensitivity of the EC GLC system for Kepone.

2. Care must be taken to ensure that the final solution contains ca. 1-2% methanol.

VI. ANALYSIS OF WATER:

1. Using a 50 ml graduated cylinder, transfer 50 ml of vigorously shaken water sample into a 125 ml separatory funnel and add 5 ml of pesticide grade benzene.

2. Stopper and shake the flask for 2 minutes, let the layers separate, and drain the water (lower) layer back into the 50 ml cylinder.

3. Percolate the benzene portion through a small amount of granular sodium sulfate into a 15 ml centrifuge tube.

4. Transfer the water in the cylinder back into the separatory funnel, rinsing the cylinder with two portions of 2.5 ml each of benzene and collecting these rinses in the separatory funnel.

5. Repeat Steps 2 and 3 once more and then discard the water layer.

6. Concentrate the combined benzene extract in the centrifuge tube under a gentle stream of nitrogen at ambient temperature to a volume appropriate for EC GLC, adjusting the final solution to contain a concentration of 1-2% methanol.

7. If injection of the sample indicates need for cleanup of the extract, proceed with micro Florisil column chromatography as described above for the analysis of blood. Base partitioning was found unnecessary in our laboratory for the water samples analyzed. If it should be considered necessary as an adjunct to the micro Florisil chromatography, it would be carried out at this point as follows:

   a. Evaporate the sample extract just to dryness under a gentle stream of nitrogen.

   b. Add 10 ml of hexane and 10 ml of 5% aqueous sodium hydroxide solution to the tube containing the sample.

   c. Vortex mix the sample for about 30 seconds and let the phases separate.

   d. Discard the hexane layer, and extract the aqueous alkali solution with at least two 10 ml portions of diethyl ether or until the aqueous phase remains clear.

   e. Transfer each ether extract with a disposable pipet and combine in a 50 ml centrifuge tube.

f. Evaporate the ether just to dryness under a stream of nitrogen, and dissolve the residue in an appropriate amount of benzene containing 1-2% methanol.

g. Determine Kepone by injection of 5 μl of solution into the EC GLC.

NOTES:

1. Tests with spiked samples indicate that recoveries of Kepone by the sodium hydroxide partitioning method approximate 90%.

2. Potassium hydroxide cannot be substituted for sodium hydroxide.

3. Standards and samples stored in solutions containing methanol require hydrolysis of the extract with 2 ml of 2 N hydrochloric acid at 80°C for 1 hour before base partitioning in order to minimize losses of Kepone partitioned into hexane.

VII. ANALYSIS OF SEDIMENT, SOIL, AND SLUDGE:

1. After thawing, mix the sample well and air dry on a large watch glass.

2. Soxhlet extract 20 grams of air dried (24-48 hours) sample for 16-18 hours with 300 ml of methanol-benzene (1:1 v/v) solvent.

3. Attach a 3-ball Snyder column to the boiling flask and reduce the volume of extract to ca 75 ml.

4. Quantitatively transfer the extract to a 100 ml volumetric flask and dilute to volume with benzene.

NOTE: Samples with Kepone levels below 0.5 ppm usually require cleanup by Florisil column chromatography (Subsection V,9) and/or by base partitioning (Subsection VI,7).

VIII. ANALYSIS OF AIR:

1. Remove a portion (ca. 60 mm x 60 mm) of the Hi-Vol sample paper, representing ca. 1/12 of the total collected sample.

2. Extract by shaking in a 125 ml Erlenmeyer flask for 5 minutes with 100 ml of methanol-benzene (1:1 v/v). A 50 ml screw cap centrifuge tube with 50 ml of solvent may be used as an alterna-

tive for the extraction.

3. Filter the sample extract through Whatman No. 1 filter paper in a Buchner funnel to remove the glass fiber.

4. Concentrate the extract to an appropriate volume for EC GLC. If the GLC scan indicates a high level of background interference, it will be necessary to conduct a micro Florisil cleanup (Subsection V,9), particularly if the Kepone level is below 1 ng per cubic meter of air.

IX. ANALYSIS OF FINFISH:

1. Remove the entrails of fish and handle as a separate sample by the technique given below. Prepare a homogeneous fish tissue sample with a Hobart food chopper. If necessary, store the fish tissue in a freezer at -10°C until the time of analysis.

2. Grind a 25 gram subsample in a 500 ml mortar with sufficient anhydrous sodium sulfate (ca. 100 grams) to dry the sample.

3. Transfer the sample to a pre-extracted thimble and Soxhlet extract with 300 ml of diethyl ether-petroleum ether (1:1 v/v) for 12-16 hours.

4. Replace the extractor tube with a Snyder column, and concentrate the extract to approximately 50 ml using the same heating mantle as during the extraction.

5. Transfer the concentrated extract to a 100 ml volumetric flask using benzene-methanol (99:1 v/v).

6. Make a screening injection into the gas chromatograph to determine if micro Florisil column cleanup is necessary. If so, proceed as follows:

   a. Prepare Florisil columns as described in Subsection V,9, a and b.

   b. Substitute 10 ml of petroleum ether for methanol-benzene-hexane (2:4:94 v/v) as the wash solvent.

   c. Apply 0.5 ml of sample solution to the column, equivalent to 500 mg of original sample.

   d. Rinse the sample tube with two 0.5 ml portions of Solvent I (25 ml of petroleum ether).

e. Add these two rinsings and the rest of Solvent I to the column and collect as Fraction I in a 50 ml centrifuge tube.

f. Elute the column with 40 ml of Solvent II (methanolacetonitrile-benzene-hexane, 1:2:4:93 v/v) and collect in a 100 ml centrifuge tube. This fraction should contain ca. 80% or more of the Kepone.

g. Adjust Fraction II to an appropriate volume by nitrogen blowdown or steam temperature solvent evaporation, being sure that the final injection solvent consists of at least 1% methanol in benzene.

NOTES:

1. An alternate extraction procedure consists of extraction of a homogenate with 25 ml of toluene-ethyl acetate (1:3 v/v) using a Polytron tissue homogenizer.

2. Additional or alternative cleanup procedures for fish extracts can include base partitioning and/or gel permeation chromatography.

X. ANALYSIS OF FINFISH LIVERS AND ENTRAILS:

1. Homogenize large samples in a Sorvall Omni-Mixer at high speed with acetonitrile for ca 2 minutes. When only a small amount of sample is available, macerate the sample (ca 500 mg) in a motor driven Duall tissue grinder with 2.5 ml of acetonitrile.
2. Separate the macerated tissue from the solvent by centrifugation.
3. Remove the solvent by pipet and place in a 50 ml centrifuge tube.
4. Repeat the maceration/extraction twice, combining the extracts in the same centrifuge tube.
5. Mix the combined extract with 25 ml of 2% aqueous Na_2SO_4 and partition against 5 ml of benzene.
6. Repeat the extraction twice more with 2 ml portions of benzene.
7. Combine the three benzene extracts in a 10 ml concentrator tube and evaporate to 0.1 ml with a gentle stream of nitrogen.
8. Wash the walls of the tube with an additional 0.5 ml of benzene and concentrate by the same procedure to 0.1 ml.

9. If a screening injection into the gas chromatograph indicates the need for micro Florisil column cleanup, follow Subsection IX, 6, a-g, exactly.

XI. ANALYSIS OF SHELLFISH:

1. Approximately six clams or oysters are thawed, shucked, and drained. Homogenize the composited meat sample in a blender at high speed for 2 or 3 minutes. If the sample is to be stored before analysis, transfer to a glass container and place in a freezer at -10°C.

2. Blend a 10-30 gram subsample of the homogenate with 50 ml of acetonitrile at high speed in a blender.

3. Vacuum filter the extract through sharkskin paper, without making any attempts to remove the solid particulate matter on the sides of the blender.

4. Scrape the sides of the blender jar with a spatula, allowing the solid material to fall to the bottom of the jar.

5. Perform a second extraction with an additional 25 ml of acetonitrile and pass the extract through the same filter.

6. Transfer the combined extracts to a 1 L separatory funnel containing 300 ml of distilled water, 5 ml of aqueous saturated Na_2SO_4, and 50 ml of benzene, and shake for 2 minutes.

7. Discard the aqueous layer and wash the benzene layer twice with 50 ml of distilled water.

8. Pass the extract through a funnel containing ca 5 grams of anhydrous Na_2SO_4, concentrate by nitrogen blowdown, and make an exploratory injection into the gas chromatograph.

9. If micro Florisil column cleanup is indicated, follow Subsection IX,6 with 25 ml of petroleum ether as Solvent I, 12 ml of benzene followed by 12 ml of methanol-benzene (1:9 v/v) as Solvent II, and 24 ml of methanol-benzene (9:9 v/v) as Solvent III. Fractions II and III contain the eluted Kepone from the column.

10. Fractions II and III are analyzed separately. If Fraction III contains more Kepone than Fraction II, it is possible that more Kepone can be recovered from the column by another elution with Solvent III. If time is crucial, Fractions II and III can be combined and analyzed as one fraction. Figure 1 illustrates chromatograms of the various micro Florisil column fractions

from a shellfish sample. A sample co-extractive having the same retention time as Kepone can be seen in the Fraction I chromatogram (A).

XII. GAS CHROMATOGRAPHY:

1. The parameters of carrier gas flow rate and column temperature depend on the column selected. If one assumes that a column of 1.5% OV-17/1.95% OV-210 is used, typical parameters would be as follows:

Column temperature	200°C - 210°C
Nitrogen flow rate	60-80 ml/minute
Tritium EC detector	210-215°C, DC mode at 80-85% standing current, linear range up to 100 pg of Kepone injected
^{63}Ni detector	250°C, DC mode at 90% standing current, linear range up to 100 pg of Kepone injected
^{63}Ni linearized detector	1 na, argon containing 5% methane carrier gas flow rate 80 ml/minute, purge gas 10-20 ml/minute, linear response up to 500 pg of Kepone injected
Inlet	235°C
Transfer line	220°C

2. Determine the Kepone concentration at an appropriate quantitative screening level (e.g., 5 ppb for blood) with a suitable column and electron capture detection. All samples and standards should contain 1-2% methanol to obtain maximum EC response.

NOTE: Methanol has been found to enhance and stabilize the electron capture gas chromatographic response of Kepone dissolved in nonpolar solvents. The response reached a maximum at approximately 1-2% methanol content; an increased percentage of methanol did not elevate the response. This was apparently due to the formation of the corresponding hemiketal, which is less likely to adhere to surfaces in a microliter syringe. It was found that Kepone could not be removed from a syringe when injected in hexane or benzene containing no methanol. Solvents which allowed maximum response without the presence of methanol were: acetone, ethyl acetate, and toluene-ethyl acetate (1:3 v/v).

3. Confirm the presence of Kepone in all samples above this level on both OV-17/OV-210 and the OV-1 GLC columns or other alternative, equivalent columns. The relative retention times of Kepone on several liquid phases are listed in the following table:

Liquid phase	RRT*	Oven temp. (C)	Carrier flow rate (minimum)
3% OV-1	2.91	180	70
	2.81	200	70
5% QF-1	2.65	180	45
5% OV-210	2.61	180	60
4% SE-30/6% OV-210	2.58	200	70
1.5% OV-17/1.95% OV-210	2.85	200	60

*relative to aldrin

4. Samples containing 5-50 ppb levels of Kepone may be further confirmed by chemical derivatization and are treated as described in Subsection XII.

5. Additional confirmation can be obtained by use of the more specific Hall conductivity GLC detector system and GLC-MS in the chemical ionization mode with continuous monitoring of four ions in the quasi molecular region (Miscellaneous Notes 1 and 2).

XIII. RECOVERY AND RESPONSE:

1. The choice of solvents is critical in the extraction, cleanup, and analysis for Kepone. Also, unless great care is taken to exclude water from solvents, the compound exists in solution as the hydrate. Aliphatic hydrocarbon solvents such as hexane are poor solvents for this pesticide; methanol, acetone, and benzene are better solvents. At ambient temperature methanol

apparently reacts with Kepone to form the hemiketal. Combined gas chromatography-mass spectrometry (GLC-MS) indicates that the hydrate and hemiketal forms revert to Kepone in the injection port of a gas chromatograph at temperatures above 200°C. Due to the polar nature of Kepone, it was difficult to remove it from substrate using nonpolar extraction solvents such as petroleum ether or hexane. In general, the most effective extraction solvent systems were methanol-benzene (1:1 v/v) and toluene-ethyl acetate (1:3 v/v).

2. Kepone does not quantitatively elute from some of the Florisil columns used in the standard multiresidue methods. For example, the Mills, Onley, Gaither macro Florisil system (Subsection 5,A,1) only partially elutes Kepone (< 8% recovery). A further problem with this system is that the Kepone elutes in both the 15 and 50% fractions. The recovery of Kepone through the micro Florisil column system listed for chlorinated pesticides in human or animal tissue and human milk (Subsection 5,A,2) is not quantitative. Minor modification of the solvent system (replacement of hexane by benzene) allows for good separation with good quantitative recovery. The solvent systems presented in these methods allow the best possible removal of co-extractives from extracts, while permitting acceptable recoveries of Kepone.

3. The limit of quantification of the method for Kepone was ca 5 ppb in blood, 40 ppt in water, 0.1 ng/m^3 in air, and 10 ppb in sediment, soil, sludge, fish, and shellfish.

4. The completeness of extraction of Kepone from all sample types was validated by exhaustive extraction of the sample with additional solvent systems and by comparison with spiked standard reference samples for the specific substrate. Recovery of Kepone from blood at 5-10 ppb was generally greater than 85%. At Kepone levels below 5 ppb, it is very difficult to assign a quantitative value with any degree of confidence. This uncertainty is caused by interferences from co-extractives and a greater variability in recovery at lower levels. For these reasons, the lower practical limit of reporting for blood is 5 ppb, and samples below this level are reported as nondetectable.

5. Recovery of Kepone from spiked water samples was generally 90% or greater.

6. The recovery of Kepone from sediments and soil exceeded 90%. Additional extraction of soil and sediment samples after acidification yielded no significant (< 1%) increase in Kepone.

7. Typical extraction efficiency of Hi-Vol air filters spiked with a total of 1 µg of Kepone was greater than 90%.

8. Extraction and cleanup efficiencies for Kepone in fish were in the range of 85-95%, depending on the specific method variation. Recoveries of Kepone standards and spiked fish samples, using the gel permeation cleanup method, were greater than 90%.

9. Typical overall recovery after extraction and cleanup of shellfish spiked at 0.6-0.8 ppm was approximately 80%.

XIV. CONFIRMATION DERIVATIZATION:

The procedure for qualitative confirmation of Kepone by chemical derivatization is as follows:

1. Transfer the cleaned up sample extract (Florisil column Fraction II) to a 16 mm x 77 mm screw-cap culture tube.

2. Evaporate the solvent just to dryness under a gentle nitrogen stream.

3. Add ca 200 mg of anhydrous, reagent grade phosphorus pentachloride, 50 mg of anhydrous, reagent grade aluminum chloride, and 3.0 ml of carbon tetrachloride to the tube, close with a Teflon-lined screw cap, and place in a heating block at 145°C for 3 hours.

NOTES:

1. At this point, reagent blanks and Kepone standards in approximately the same amount as is expected in the sample extracts should be derivatized in parallel with samples.

2. The presence of aluminum chloride in the reaction mixture improves the completeness and reproducibility of the derivative formation. The mode of action of aluminum chloride is not yet known.

3. Remove the tube, cool to room temperature, and open.

4. Add 3 ml of distilled water, reclose the tube, and shake for 2 minutes.

5. After phase separation, transfer 2.0 ml of the lower carbon tetrachloride layer to a clean 5 ml graduated centrifuge tube, using a disposable pipet.

6. Evaporate the solvent in the 5 ml tube just to dryness under a stream of nitrogen.

7. Using a disposable pipet, transfer the residue to a micro Florisil column (Subsection V,9) with the aid of three 0.5 ml portions of hexane.

   NOTE: Florisil cleanup is necessary when the total amount of Kepone is less than 25 ng. When more than 200 ng is present, the derivatized extract can be analyzed without Florisil cleanup. In this case, take special care to evaporate all the carbon tetrachloride before dissolving the residue in hexane for injection into the electron capture gas chromatograph.

8. Elute the mirex from the column with an additional 8.5 ml of hexane, collecting the total effluent in a 15 ml centrifuge tube.

   NOTE: Any mirex present in the original sample would have been eluted in the first (discarded) Florisil fraction of the earlier cleanup (Subsection V,9). This ensures that mirex recovered in this step was formed from conversion of Kepone.

9. Concentrate or dilute the eluate so that a 5 μl injection into the EC GLC, operated under the conditions in Section XII, gives a mirex peak within the predetermined linear range of the electron capture detector. Note that only 2/3 of the derivatized sample extract was taken in Step 5 to be carried through the micro Florisil column cleanup.

   NOTES:

   1. The overall average percent conversion of 10-1000 ng amounts of Kepone derivatized in quadruplicate was 104%, with a relative standard deviation of 8.2%.

   2. Derivatization of other common chlorinated hydrocarbon pesticides produced no interfering GLC peaks at the retention time of mirex.

XV. MISCELLANEOUS NOTES:

1. The Hall micro electrolytic conductivity detector (Section 4,C) is operated in the oxidative chlorine mode with a furnace temperature of 830°C (nickel reaction tube), reaction gas (air) flow rate of 100 ml/minute, helium flow rate of 65 ml/minute,

and methanol conductivity solvent flowing at 0.4-0.6 ml/minute. The GLC column is 1.8 mm x 2 mm glass, containing 3% OV-101 and operated at 200°C.

2. For coupled GLC-MS, a Finnigan model 3200 GLC/MS EI-CI system with model 6000 data control system was employed in the multiple ion detection mode (MID), monitoring ions with m/e - 488.7, 490.7, 492.7, and 494.7. The operating conditions were: methane reagent carrier gas, 1000 μ ion source pressure, 80°C ionizer temperature, and 70-100 eV. A 1.8 m x 2 mm glass GLC column containing 3% OV-1 was operated at 210°C with an injection temperature of 240°C. Simultaneous monitoring of the four ions and the characteristic GLC retention time of Kepone adds substantial confidence to the identification of residues.

3. The derivatization procedure converts Kepone to mirex. The analyst should, therefore, be certain that no mirex was carried through the cleanup steps before derivatization. The micro Florisil column should elute any mirex present in the first (discarded) fraction. The absence of mirex in the Fraction II eluate can be easily established by permitting sufficient development of chromatograms to elute mirex.

4. The Autoprep 1001 GPC system (Section 5,B) was used for the additional removal of lipids from fish tissue extracts with the following parameters before Florisil column chromatography.

Column	230 mm x 25 mm i.d., packed with 200-400 mesh Bio-Beads SX-3
Solvent	toluene-ethyl acetate (1:3 v/v)
Pumping rate	3.5 ml/minute
Discard volume	0-72 ml
Collect volume	73-113 ml
Wash volume	114-154 ml

Recovery of Kepone standards through GPC averaged about 95%. Recovery of Kepone from actual fish samples at 0.1 ppm levels ranged from 80-86% after GPC plus Florisil column cleanup.

XVI. ANALYTICAL QUALITY CONTROL:

1. Sufficient control and spiked standard reference materials should be used to assure the validity of analytical results.

2. Elution patterns for Florisil columns should be carefully established by each analyst. These can vary appreciably in different areas and even to some extent between analysts in the same laboratory.

3. Analytical standards should be validated by cross-reference analysis of additional preparations of analytical grade Kepone with agreement within ±5% of the established purity.

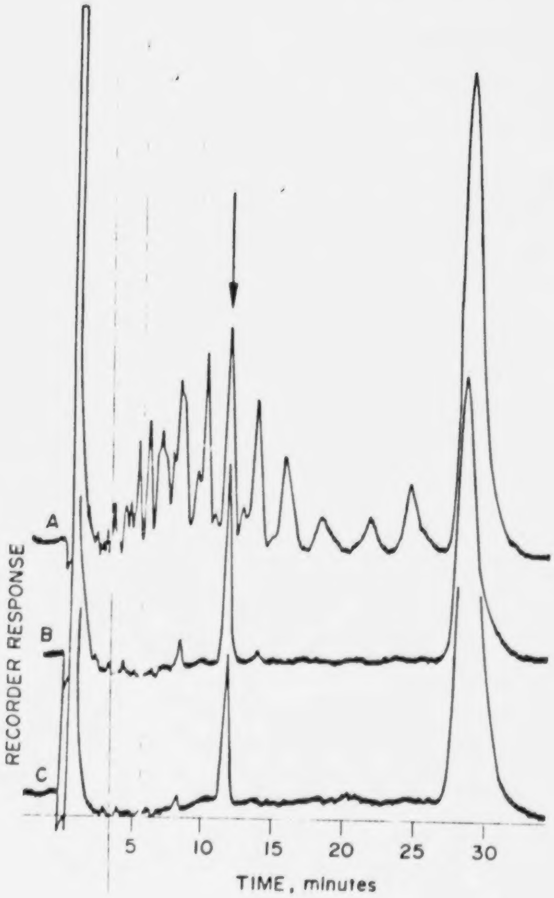

Figure 1. Chromatogram of shellfish micro Florisil fractions: A. Fraction I (no Kepone elution in this fraction); B. Fraction II; and C. Fraction III. Injection of 100 µg equivalent of shellfish. Column: 3% OV-1, nitrogen flow rate: 60 ml/minute.

CLEANUP BY GEL PERMEATION CHROMATOGRAPHY (GPC)

I. INTRODUCTION:

Gel permeation chromatography is useful for the cleanup of biological extracts for residue analyses of low molecular weight organic compounds and pesticides. Large molecules are excluded from the pores of the gel and elute first. Later fractions containing low molecular weight organic compounds of interest can be collected and analyzed by analytical chromatography, such as GLC or HPLC. The self-regeneration characteristics of GPC allows sequential sample analyses on the same column and consequently automation. GPC adds another dimension to cleanup by using molecular size instead of partition and adsorption coefficients, which in some cases may not be favorable.

The original gel permeation chromatography (GPC) system using BioBeads SX-2 crosslinked polystyrene gel (EPA Pesticide AQC Manual, Section 7,M) has undergone extensive revision by the authors and other investigators. The automated system has been commercially available from Analytical BioChemistry Laboratories, Inc., Columbia, MO, since 1974 (Figure 1).

The first GPC applications involved the separation of fish lipid and nonionic chlorinated hydrocarbons from fish tissue desiccated with anhydrous sodium sulfate and extracted with 6% diethyl ether and petroleum ether. Mobile phases composed of toluene-ethyl acetate (1:3 v/v), ethyl acetate, methylene chloride, and mixtures of methylene chloride-cyclohexane containing from 5 to 50% methylene chloride have been found useful for cleanup of pesticides and other organic pollutants from plant and animal tissue extracts. Recently, Biobeads SX-3 has been used by some workers in place of SX-2.

Biobeads SX-3 eluted with toluene-ethyl acetate (1:3 v/v) has been successful in removing lipid interferences from fish extracts for subsequent electron capture GLC analysis of Kepone. If PCB, dieldrin, or endrin interferences were observed by EC GLC, the extract was sufficiently lipid free to allow reproducible liquid adsorption column chromatography to isolate Kepone (Section 5,A,(5),(a)). A similar system was described by Fehringer for the analysis of polybrominated biphenyl residues in dairy products.

Hopper and Hughes found that methylene chloride-cyclohexane (10:90 v/v) resulted in increased resolution of lipids and pesticides observed in a total diet survey composed of fatty products such as butter, mayonnaise, shortening, and margarine. Pesticides monitored included nonionic chlorinated hydrocarbons, organophosphates, and carbamates. Kuehl and Leonard, from the U. S. EPA, Duluth, Minnesota Environmental Research Laboratory, investigated various solvent mixtures of methylene chloride and cyclohexane ranging from 100% methylene chloride to 10% methylene chloride-cyclohexane. The 50:50 v/v mixture of methylene chloride-cyclohexane gave the best resolution of lipids and polar-nonpolar low molecular weight organics for gas-liquid chromatographic-mass spectroscopic analyses. Recoveries were demonstrated on 27 compounds, including Aroclors, HCB, DDT, and PCP. Fifty-seven compounds were identified in fish tissue by fractionation and GLC MS analysis.

Crist and Moseman (Sections 4,C,(3) and 12,A) used gel permeation chromatography for additional cleanup of certain human biological extracts having an adverse effect on the performance of the Hall detector due to excessive lipid material.

Investigation by Shofield et al. revealed the cleanup capability of GPC used in conjunction with crop, vegetable, and fruit extracts for organophosphates. Satisfactory recoveries were observed for parent compounds as well as their oxidized analogs. The retention volumes of alkyl and aryl organophosphates could be decreased by increasing the percentage of methylene chloride in cyclohexane, Leight et al. reported the applicability of GPC to cleanup or organophosphate, triazine, and carbamate pesticide residues. Pflugmacher and Ebing have done extensive elution profile characterization on various gels and elution solvents and have typified 106 pesticides including organophosphates, carbamates, thioureas, triazines, phenoxy acids, and phenoxy acid esters. Fujie and Fullmer determined 0.05 ppm residues of a new synthetic pyrethroid insecticide in samples containing oil and lipid by EC GLC after GPC and Florisil column cleanup.

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II. ELUTION PATTERNS AND RECOVERY DATA FOR PESTICIDES:

The data in the following table consists of elution profiles for some common pesticides from the AutoPrep 1001 gel permeation chromatograph. All data are from a 50 gram, 30 x 2.5 cm column of BioBeads SX-3 gel, with a flow rate of approximately 5.0 ml/minute unless otherwise noted.

The elution profiles were compiled by personnel at Analytical BioChemistry Laboratories, Inc. To be certain of maximum recovery and accurate quantitation of samples, analysts should calibrate their particular columns under local conditions with standards of the pesticides of interest.

The following symbols are used throughout the Table:

( ) - Method of detection:

1. GLC/Electron Capture
2. GLC/Sulfur-Phosphorus Emission Detector
3. High Pressure Liquid Chromatography/UV Detector
4. GLC/Alkali Flame Ionization Detector

* - Not fractionated but recovered quantitatively within these parameters

** - GPC column - 30 grams, 2.5 x 17.5 cm column of BioBeads SX-3

N.T.- Not Tested

Nonionic Chlorinated Compounds	Elution Volume (ml) with Methylene Chloride-Cyclohexane (15:85)	Elution Volume (ml) with Toluene-Ethyl Acetate (1:3)
1. Aldrin [1]	110→150	110→130
2. α-BHC [1]	150→190	90→130
3. α-Chlordane [1]	120→170	100→120
4. γ-Chlordane [1]	120→170	100→120
5. p,p'-DDD [1]	160→210	90→120
6. p,p'-DDE [1]	110→160	110→160
7. o,p'-DDT [1]	110→150	110→130
8. p,p'-DDT [1]	120→170	110→130
9. Dieldrin [1]	140→180	110→130
10. Endrin [1]	130→170	110→130
11. Heptachlor [1]	110→150	100→130
12. Heptachlor Epoxide [1]	120→170	110→130
13. Hexachloro Benzene (HCB) [1]	120→160	120→150
14. Lindane [1]	160→210	100→130
15. Methoxychlor [1]	140→200	100→120
16. Mirex [1]	90→140	110→130
17. Toxaphene [1]	120→240*	100→140
PCBs and PBBs		
1. Arochlor 1016 [1]	120→240*	N.T.
2. Arochlor 1242 [1]	120→240*	110→140
3. Arochlor 1254 [1]	120→240*	100→150
4. Arochlor 1260 [1]	120→240*	110→140
5. PBB (hexa) [1]	N.T.	140→180
Organophosphates		
1. Acephate [2]	200→320	N.T.
2. Azodrin [4]	150→240	N.T.
3. Dasanit (PSSO) [2]	140→230	80→110
4. Dasanit (POSO) [2]	140→220	N.T.
5. Dasanit ($POSO_2$) [2]	160→250	N.T.
6. Dasanit ($PSSO_2$) [2]	160→250	N.T.
7. DDVP (Vapona) [2]	100→200	90→120
8. Diazinon [2]	80→160	90→120
9. Dimethoate [2]	140→240	N.T.
10. Dioxathion [2]	140→190	N.T.
11. DiSyston (Parent-PSS) [2]	110→160	100→130
12. DiSyston (POS) [2]	110→160	N.T.
13. DiSyston (PSSO) [2]	100→160	N.T.
14. DiSyston ($PSSO_2$) [2]	160→210	N.T.
15. Dursban [2]	120→210	N.T.
16. Dyfonate [2]	130→210	N.T.
17. Ethion [2]	110→160	N.T.
18. EPN [2]	150→220	N.T.

	Elution Volume (ml) with Methylene Chloride-Cyclohexane (15:85)	Elution Volume (ml) with Toluene-Ethyl Acetate (1:3)
19. Fenthion [4]	130→200	N.T.
20. Gardona [2]	120→180	N.T.
21. Malathion [2]	120→200	90→110
22. Methamidophos [4]	140→260**	N.T.
23. Methidathion [2]	120→240	N.T.
24. Naled [2]	120→190	N.T.
25. Paraoxon [2]	155→235	N.T.
26. Parathion (Ethyl) [2]	120→230	90→120
27. Parathion (Methyl) [2]	190→250	90→120
28. Phosdrin [2]	140→210	N.T.
29. Pirmiphos (Methyl) [2]	100→160	N.T.
30. Ronnel [2]	130→210	N.T.
31. Ruelene [2]	120→210	N.T.
32. Thimet [2]	110→170	N.T.
33. Trithion [2]	140→180	N.T.
Carbamates		
1. Aminocarb [3]	140→210	N.T.
2. Carbaryl [3]	210→280	N.T.
3. Carbofuran [3]	160→220	N.T.
4. Carbofuran, 3-keto [3]	120→230	N.T.
5. Carbofuran 3-OH [1]	240→310	N.T.
6. Captafol [3]	160→260	N.T.
7. Mesurol [3]	170→220	N.T.
8. α-Napthol [3]	290→350	N.T.
9. Zectran [3]	130→200	N.T.
Thioureas		
1. Cotoran [3]	160→230	N.T.
2. Diuron [3]	210→280	N.T.
3. Fenuron [3]	160→240	N.T.
4. Monuron [3]	200→280	N.T.
Synthetic Pyrethriods		
1. Permetrin (Both Isomers) [1]	130→190	N.T.
2. Pydrin [1]	110→170	N.T.
Triazines		
1. Atrazine [1]	140→200	N.T.
2. Bladex [1]	150→300	N.T.
3. Simazine [1]	160→210	N.T.

	Elution Volume (ml) with Methylene Chloride-Cyclohexane (15:85)	Elution Volume (ml) with Toluene-Ethyl Acetate (1:3)
Other Chlorinated Compounds		
1. Kepone [1]	N.T.	100→190
2. 2,4-D Esters:		
Isopropyl [1]	N.T.	90→120
Butyl [1]	N.T.	90→120
PGB [1]	N.T.	80→110
Butoxyethanol [1]	N.T.	80→120
Iso Octyl [1]	N.T.	80→120
Ethyl Hexyl [1]	N.T.	80→120
Methyl [1]	N.T.	90→130
3. Silvex Esters:		
Propylene Glycol [1]	N.T.	N.T.
Methyl [1]	N.T.	N.T.
4. 2,4,5-T Esters:		
Isopropyl [1]	N.T.	90→130
Butyl [1]	N.T.	90→130
Ethyl Hexyl [1]	N.T.	90→130
Iso Octyl [1]	N.T.	90→120
Methyl [1]	N.T.	90→130
Miscellaneous Compounds		
1. Metribuzin (parent)	110→160**	N.T.
2. Metribuzin (DA)	100→170**	N.T.
3. Metribuzin (DADK)	140→200**	N.T.
4. Metribuzin (DK)	140→180**	N.T.
5. Treflan	100→140	90→110

III. CLEANUP OF ADIPOSE TISSUE:

See Section 8,M,j of the EPA Pesticide AQC Manual for a description of the theory, equipment, column preparation and operation, and procedure for the GPC cleanup of adipose tissue using toluene-ethyl acetate (1:3 v/v) as the mobile phase. This procedure has, for example, been used in the EPA HERL laboratory for cleanup in the identification of polychlorinated terphenyls in human adipose tissue at trace levels by GLC MS (Wright, L. H., Lewis, R. G., Crist, H. L. Sovocool, G. W., and Simpson, J. M., J. Anal. Toxicol., 2, 76 (1978)).

J. A. Ault of Analytical BioChemistry Laboratories has described the use of cyclohexane-methylene chloride (85:15 v/v)/ BioBeads SX-3 GPC cleanup coupled with a deactivated mini-alumina column for blended feed-grade fat samples containing low molecular weight fatty acids and glycerides. In this improved procedure, the GPC eluate as it emerges from the column is directed through a 1.5 gram column of alumina (deactivated with 5% water) in the neck of a powder funnel.

The preparation of the powder funnel/alumina column (Figure 2) is as follows:

1. Place a plug of glass wool in the end of the powder funnel neck and rinse with 5 ml cyclohexane-methylene chloride (85:15 v/v).
2. Pour approximately 1.5 grams of 5% water-deactivated alumina (v/w) into the funnel.

The reservoir of the powder funnel must be large enough to hold approximately 100 ml of solvent because the alumina restricts solvent flow. After all solvent has passed through the funnel, the alumina should be rinsed with 15 ml of cyclohexane-methylene chloride (85:15 v/v) to wash any remaining residues into the collection vessel. The samples are rotary vacuum evaporated, transferred to culture tubes with 5 ml of petroleum ether, air-stream evaporated to 1 ml, and analyzed by EC GLC.

Figures 3 and 4 compare gas chromatograms of a pesticide-containing fat sample prepared by GPC alone with that treated by GPC plus alumina. Figure 3 shows interferences from lipids and phthalates, while the modified procedure chromatogram (Figure 4) indicates adequate cleanup to quantitate chlorinated pesticides. The allows mark peaks that correspond to 0.08 ppm dieldrin and 0.12 ppm endrin, respectively, which match the values obtained from a previous independent analysis of this same sample. Average recovery of 16 compounds was 89% using this new technique, with percent coefficient of variation of lindane, p,p'-DDE, and dieldrin of 8%, 10%, and 7%, respectively.

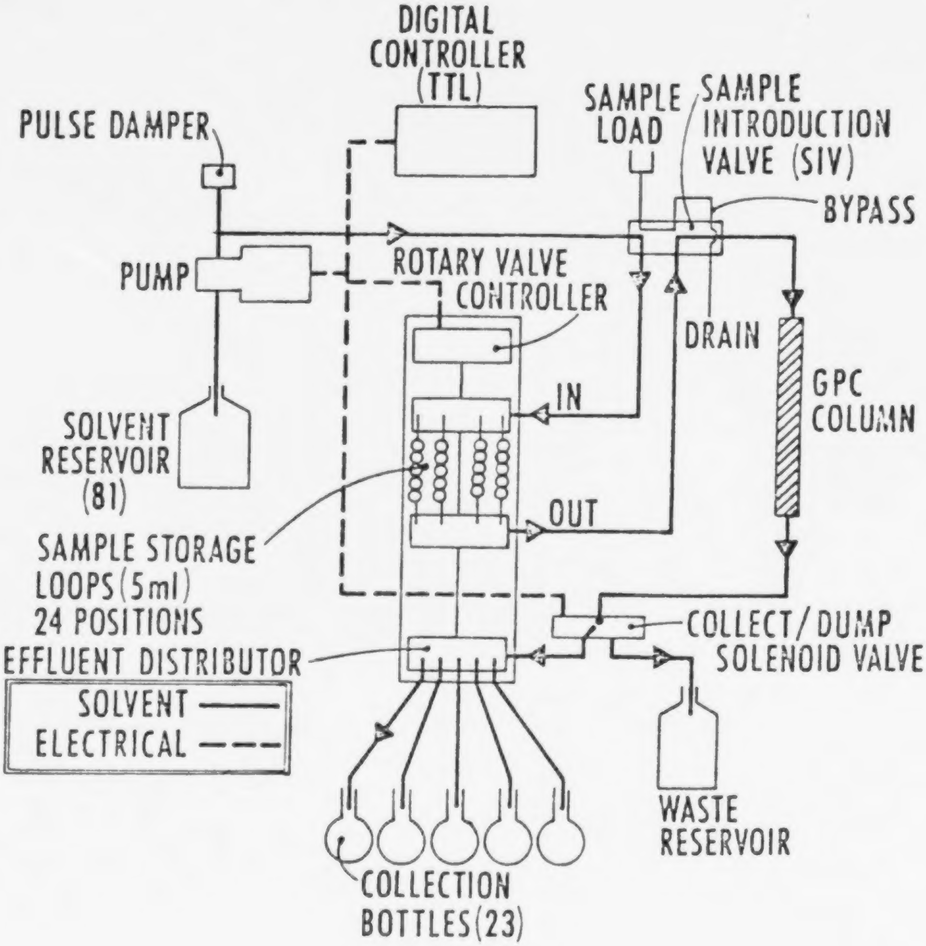
DIGITAL CONTROLLER (TTL)
PULSE DAMPER
SAMPLE LOAD
SAMPLE INTRODUCTION VALVE (SIV)
BYPASS
PUMP
ROTARY VALVE CONTROLLER
DRAIN
GPC COLUMN
IN
SOLVENT RESERVOIR (8l)
OUT
SAMPLE STORAGE LOOPS(5ml) 24 POSITIONS
COLLECT/DUMP SOLENOID VALVE
EFFLUENT DISTRIBUTOR
SOLVENT
ELECTRICAL
WASTE RESERVOIR
COLLECTION BOTTLES(23)

Fig. 1. Schematic diagram of the automated GPC AutoPrep 1001.

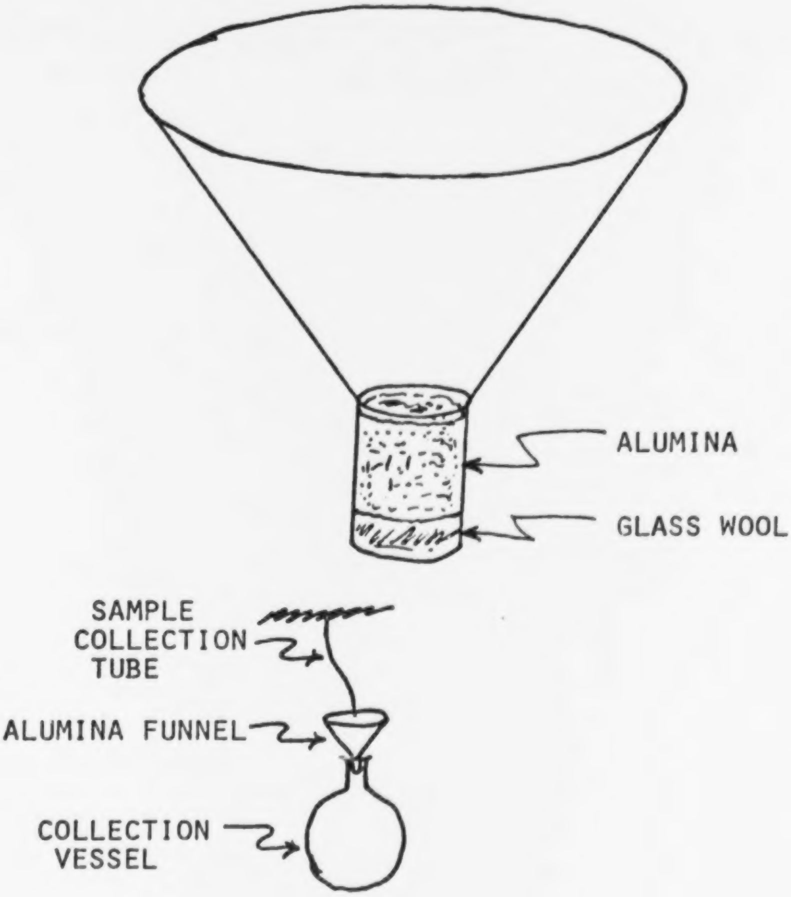
ALUMINA
GLASS WOOL
SAMPLE COLLECTION TUBE
ALUMINA FUNNEL
COLLECTION VESSEL

Fig. 2. Alumina adsorption cleanup column.

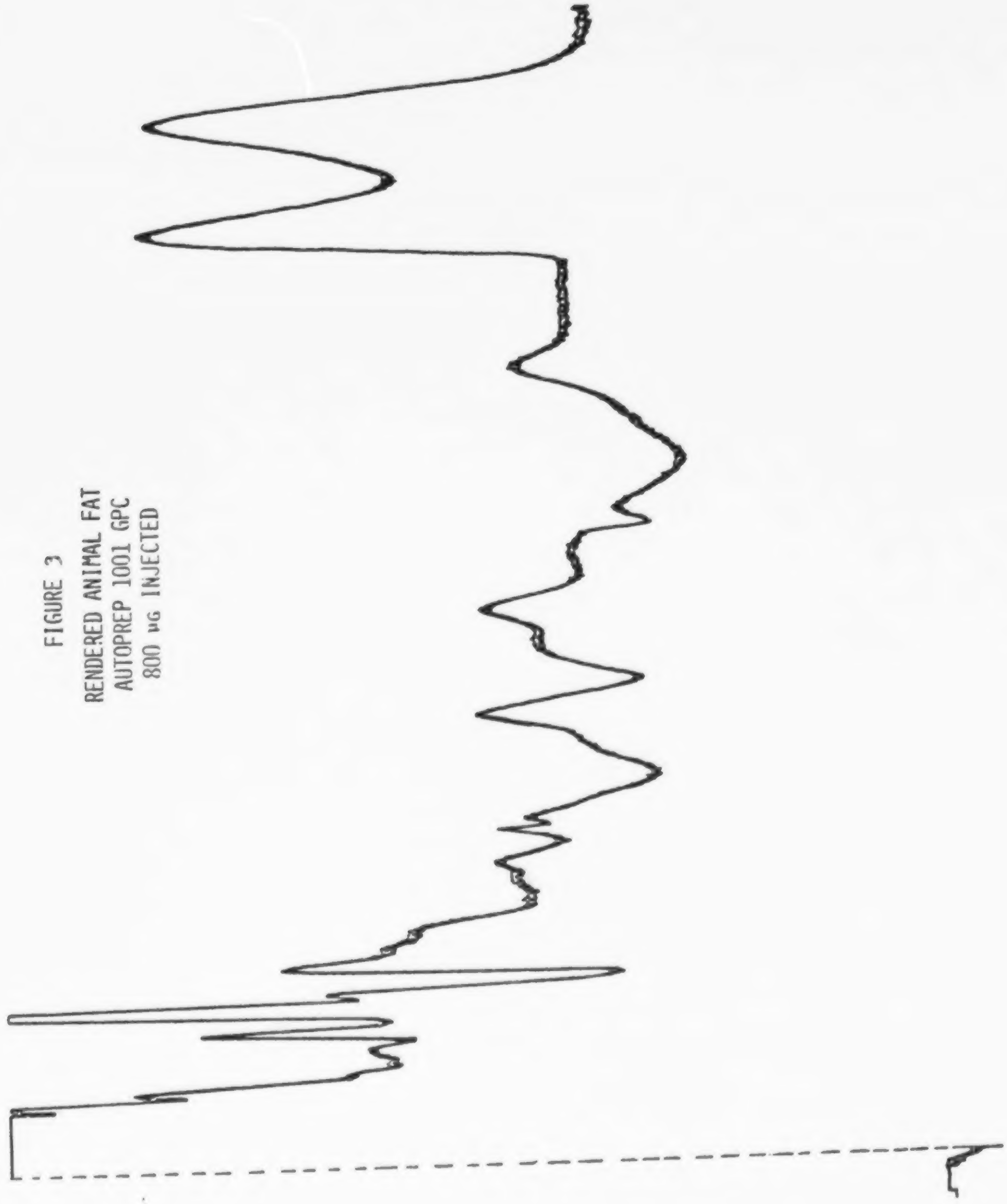

FIGURE 3
RENDERED ANIMAL FAT
AUTOPREP 1001 GPC
800 μG INJECTED

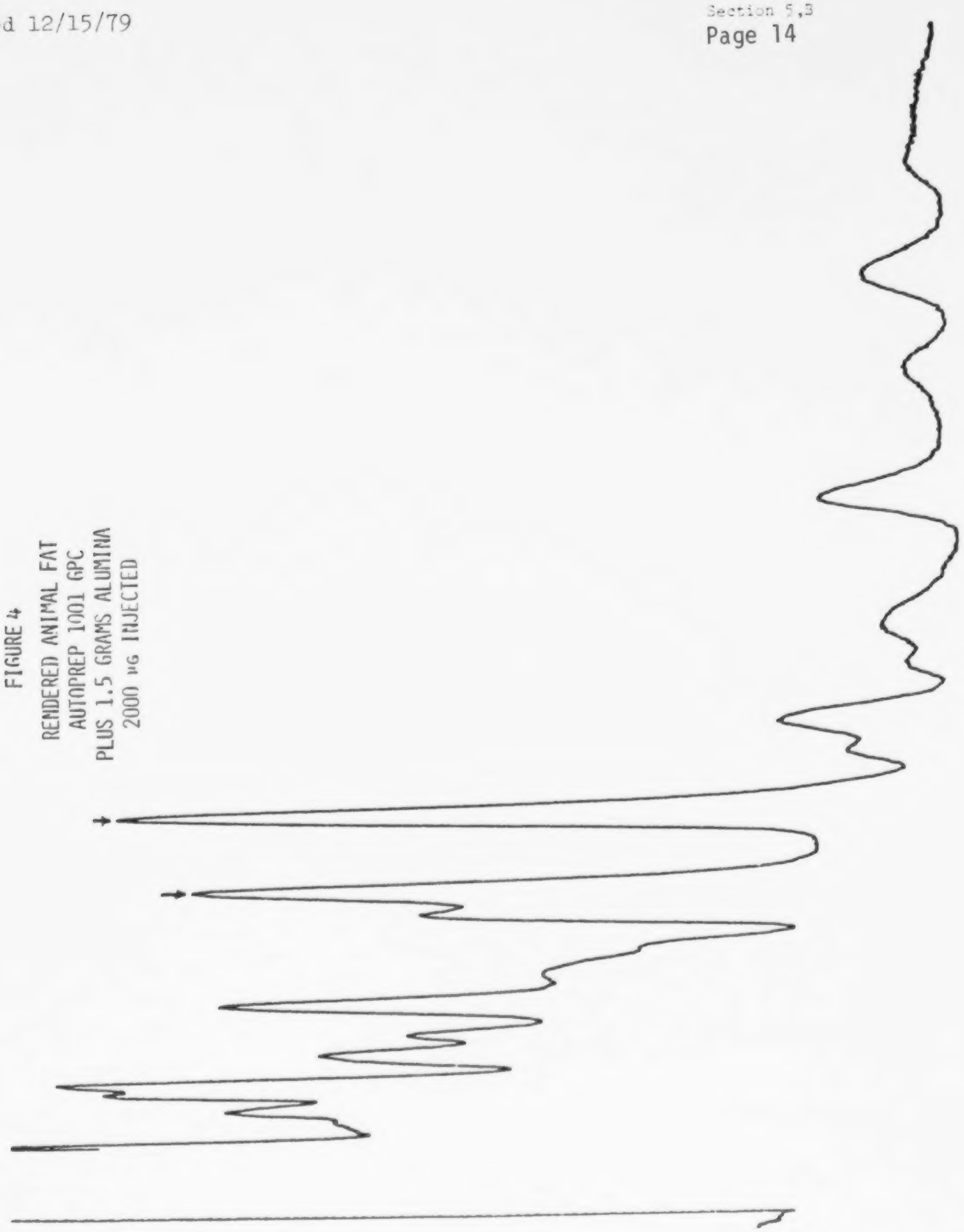

FIGURE 4
RENDERED ANIMAL FAT
AUTOPREP 1001 GPC
PLUS 1.5 GRAMS ALUMINA
2000 µG INJECTED

ORGANOPHOSPHORUS PESTICIDES AND METABOLITES IN HUMAN TISSUES AND EXCRETA - GENERAL COMMENTS AND ANALYSIS OF INTACT PESTICIDES

The pesticide residue chemist is sometimes called on to consult and perform analyses pertaining to poisoning by organophosphorus pesticides. Such cases may come about through accidental exposure, attempted homicide or suicide, or by small children ingesting the material, thinking it to be a confection.

In a suspected OP poisoning case, time is of the essence if the chemist is to obtain any of the original or parent compound that was ingested or cutaneously absorbed. The OP compounds are relatively unstable as contrasted to the organochlorine pesticides. Therefore, the chemist will find it most advantageous to work closely with the pathologist to expedite the delivery to the laboratory of tissues, gastrointestinal contents, blood, and urine so that analytical work can be started at once. In a matter of hours the parent compound, per se, may no longer be detectable in the sample substrates. The analysis of intact OP pesticides in tissue or blood from suspected poisoning victims is described at the end of this section.

The information so obtained may be sufficient to advise the medical team of the identity of the intoxicant, but the chemist may be provided with the rather rare opportunity to conduct in-depth studies of the metabolic pathways of the pesticide degradation in the patient, and to document all findings for supplementation to existing knowledge.

In cases resulting from low exposure or in high exposure cases many hours after exposure, the probability is greatly reduced of detecting the parent compound in the body fluids or tissue. The chemist is then faced with the problem of selecting appropriate body fluids for analysis, and very importantly, selecting an analytical method appropriate for the potential breakdown metabolite. A knowledge of the metabolism of these pesticides will assist the analyst in making these decisions.

The several analytical methods included in this section provide some selections for assessing exposure to the OP compounds. For example, a phenolic metabolite of methyl and ethyl parathion is para-nitrophenol. The analytical method for detection and quantitation in the urine is described in Section 6,A,(2),(b) of this section. The formation of salts of dimethyl or diethyl phosphate, thiophosphate, and dithiophosphate results from hydrolysis of various OP compounds. These metabolites appear in the urine and may be assayed by the procedure described in Section 6,A,(2),(a). The OP compounds exert an inhibiting effect on blood cholinesterase. A sensitive procedure for measuring cholinesterase activity in the blood is provided in Section 6,A,(3),(a).

The block diagram given in Appendix VI provides some guidelines for selection of methodology, not only for the OP compounds, but for other suspected exposure to pesticidal compounds.

Determination of Intact OP Pesticides in Tissue or Blood

1. Homogenize 1 gram of liver, brain, or fat with 1 ml of water. Mix the homogenate with 10 volumes of acetone and centrifuge.
2. Mix serum or whole blood with 10 volumes of acetone and centrifuge.
3. Inject either centrifugate directly into a gas chromatograph equipped with an FPD and operated as described in Section 6,A,(2),(a). If there has been significant exposure, the sensitivity will be more than adequate for detection, identification, and quantitation.
4. For the analysis of samples with low levels (< 0.1 ppm), the acetone supernatant is evaporated to 1/10 of the original volume of acetone, and column cleanup is carried out as follows:
    a. Saturate the concentrated sample with sodium chloride and extract with 15 ml of hexane.
    b. Evaporate the organic layer to 0.3-0.5 ml under nitrogen gas.
    c. Prepare a size 22 Kontes Chromaflex column packed with 2.5 cm of anhydrous Na_2SO_4 on top of 1 gram of silica gel that has been deactivated by adding 1 ml of water to each 10 grams of activated silica gel.
    d. Rinse the column of hexane and transfer the evaporated sample quantitatively.
    e. Elute the column with 10 ml of hexane (Fraction I).
    f. Elute next with 10 ml of an appropriate solvent (e.g. hexane-benzene (80:20 v/v) that will completely recover the pesticide(s) of interest (Fraction II)). The elution pattern of the column must be established with pesticide standards.

g. Evaporate Fraction II to 0.2-0.4 ml and inject a 5 μl aliquot into the FPD equipped gas chromatograph.

METHOD FOR DETERMINATION OF METABOLITES OR HYDROLYSIS PRODUCTS OF ORGANOPHOSPHORUS PESTICIDES IN HUMAN URINE, BLOOD, AND OTHER TISSUES

I. INTRODUCTION:

The metabolism and urinary hydrolysis of organophosphorus (OP) pesticides in mammals results in the excretion of a variety of alkyl phospates. These include the salts of dimethyl or diethyl phosphate and phosphorothioate, and phenylphosphonates. The gas chromatographic separation and quantification of such products in urine may be of value in estimating the extent of exposure to the parent organophosphorus pesticide. The procedure (1) permits the determination of four alkyl phosphates derived from most of the common OP pesticides and three phosphonate metabolites of the insecticide leptophos (Phosvel). Recovery data and limits of detectability for all 10 derivatives formed by these metabolites and analysis of urine samples from individuals exposed to OP pesticides are reported.

The new analytical procedure (1) is based on a previous method described in the 12/2/74 revision of this Manual (2-6). Though a small fraction of phosphonate metabolites are recovered by the acetonitrile-diethyl ether extraction step in the previous method, these compounds are recovered with ion-exchange resins from urine. The new method will, therefore, monitor phosphonate metabolites of leptophos in addition to alkyl phosphates, and additionally provide higher recoveries, decreased gas chromatographic interferences, and faster analyses than the former procedure. It has been applied with success to analysis of whole blood, serum, and other tissue samples as well as to urine.

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II. PRINCIPLE:

Organophosphate metabolites or hydrolysis products in urine are extracted quantitatively with an ion exchange resin. The metabolites are subsequently derivatized with diazopentane reagent on the resin. This reagent is used rather than the more common diazoethane because the derivatives of metabolites and interfering inorganic phosphate are more easily resolved. The derivatives are determined by gas chromatography with flame photometric detection. If very low levels of alkyl phosphate metabolites are present, further cleanup by silica gel fractionation is required to remove interfering substances such as the triamyl phosphate derivative of inorganic phosphate.

III. APPARATUS:

1. Tracor model 222 gas chromatograph with flame photometric detector operated in the phosphorus mode. The detector was equipped with a Spectrum 1020 noise filter and a variable power supply (Power Designs, Inc.). A Valco switching valve #CV 4 HT was interfaced between the GLC column and FPD detector to allow interchange of the column effluent and nitrogen purge gas (the flow rates of which should be equal to maintain a steady recorder baseline).

   NOTE: The principal purpose of the switching valve with the original FPD is to vent solvent and prevent flame blowout when injections are made. The new configuration of the FPD permits injections of >10 µl without extinguishing the flame. However, the switching valve is still used to permit operation of two different columns or to vent large peaks or column bleed. Also, the column can be silylated or Carbowax treated without running the effluent through the detector.

2. Gas chromatographic column-borosilicate glass, 1.8 m x 4 mm i.d., packed with 5% OV-210 on 80-100 mesh Gas-Chrom Q support. Prepare and condition the column by Carbowax deposition treatment as described in Section 4,B.

   NOTE: An alternative column, which may be used for confirmation of identity of peaks, is a 1.8 m x 4 mm i.d. borosilicate glass column packed with 4% SE-30/6% OV-210 on 80-100 mesh Gas-Chrom Q. Condition in a similar manner.

3. Centrifuge tubes, 13 ml capacity, conical, graduated, with ₸ ground glass stoppers.

4. Pipets, disposable glass, Pasteur type, 9 in. length, fitted with rubber bulbs.

5. Pipets, disposable glass, 5 ml capacity, for use as ion exchange columns.

6. Vortex-Genie mixer.

7. IEC centrifuge, Model EXD, explosion proof, operated at 2000 rpm.

8. Culture tubes, glass, 16 x 150 mm.

9. Pipet, 0.1 ml capacity, graduated in 0.01 ml units.

10. Pipets, assorted capacities, to be used in combination with appropriate volumetric flasks for preparation of standard solutions.

11. Bottles, reagent, narrow mouth, 1 oz. capacity, with polyseal screw caps (A. H. Thomas 2203-C bottles and 2849-E caps).

12. Column, chromatographic, size 23 (Kontes 420100).

13. Nitrogen evaporator with water bath maintained at 40°C (organomation Associates).

14. Exhaust hood with minimum draft of 150 linear feet per minute.

IV. REAGENTS:

1. Diethyl ether, AR grade, containing 2% ethanol (Mallinckrodt 0850 or equivalent).

2. All other solvents are pesticide quality, distilled from all-glass apparatus.

3. Silica gel, Woelm, activity grade I (ICN Pharmaceuticals, Inc.), activated at 130°C for 48 hours and stored in a desiccator.

4. Potassium hydroxide, pellets, AR grade.

5. *N*-amyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.).

6. Anion exchange resin, Amberlite CG-400 AR, 100-200 mesh (Mallinckrodt 3345), in the chloride form or BioRad AG1x8, 100-200 mesh (BioRad Laboratories, Richmond, CA), in the chloride form.

7. Hydrochloric acid, reagent grade, approximately 37%.

8. Glass wool, pre-extracted with pesticide grade methylene chloride.

9. Diazopentane reagent - Preparation:

   a. Dissolve 2.3 g of KOH in 2.3 ml of distilled water in a 125 ml Erlenmeyer flask. When solution is complete, cool in a freezer for 30 minutes.

   b. Add 25 ml of cold diethyl ether, cover flask mouth with foil, and cool in a -18°C freezer for 15 minutes.

c. In a very high draft hood, add 2.1 g of N-amyl-N'-nitro-N-nitrosoguanidine to the flask in small portions over a period of a few minutes, swirling the flask vigorously after each addition.

d. Decant the ether layer into a 1 oz. reagent bottle fitted with a Teflon lined screw cap. This may be stored at -20°C for periods up to a week.

NOTES:

1. Because of the demonstrated carcinogenicity and skin irritating characteristics, do not allow the nitrosoguanidine or the diazoalkane to come in contact with the skin. Wear disposable vinyl gloves and safety goggles while handling. Avoid breathing vapors. Working inside a radiological glove box, if possible, is strongly recommended.

2. Do not use ground glass stoppered bottles or bottles with visible interior etching. Avoid strong light.

10. Standards

The names of various phosphate compounds will be abbreviated from this point on to conserve space (see Table 1). The organophosphorus potassium salts are supplied by American Cyanamid Corporation and phosphonate standards by Velsicol Chemical Corporation. Certain diethyl and dimethyl phosphate and phosphorothioate standards are available in 100 mg increments from the EPA Repository in Research Triangle Park, NC (See EPA-600/9- 76-012 for a listing of available standards).

a. Prepare stock solutions of all standards except DMP at 1 mg/ml levels in acetone. Prepare DMP at the same level in water.

b. Quantitatively make a 1:10 dilution of the stock solutions (to 0.01 mg/ml (10 μg/ml)) with acetone to prepare the working standards. These may be used for spiking control urine samples to be carried through the procedure for comparison with actual samples. To spike at 0.1 ppm, add 1 μg of compound (0.1 ml of the working standard) to 10 ml of urine; to spike at 1 ppm, add 1 ml to 10 ml of urine (see also Section XI).

c. To alkylate phosphate and phosphonate standards in the absence of urine proceed as follows:

(1) Weigh accurately 10 mg of each standard into 13 ml centrifuge tubes.

NOTE: As an alternative, pipet 0.1 ml of each working standard (equivalent to 1 μg of compound) into a 13 ml centrifuge tube, add 1 ml of acetone, and proceed with steps (2)-(5). If necessary, make further final dilutions to prepare the required standards.

(2) Add to each tube one drop of 2 M HCl to convert the salts to the corresponding free acids.

(3) Allow the standards to stand for one hour before being derivatized with diazopentane.

(4) Add sufficient diazopentane reagent to produce a persistent yellow color (2-5 ml usually suffices), mix, and allow to stand for one hour with occasional mixing of the solution.

NOTE: If, at any time during this period, the yellow color disappears, add more diazopentane.

(5) Remove excess reagent by adding a solution of formic acid-benzene (1:99 v/v) dropwise until the yellow color just disappears. Avoid an excess of this reagent.

(6) Dilute the solution(s) to exactly 10 ml with acetone, stopper, and mix thoroughly. Store in the freezer in glass stoppered containers when not in use.

d. These alkylated concentrated stock standards may be diluted individually or prepared as mixtures at an intermediary concentration range. For example, a 1:100 dilution of a concentrated standard will yield a solution of 10 ng/μl.

e. Working Standard Mixtures:

(1) Dilute each of the intermediary stock mixtures in a 1:50 ratio with acetone.

(2) To establish that the working standard mixtures are in a proper concentration range, observe the recorder response resulting from the injection of 5 μl of each

into the gas chromatograph.

Photometric tubes vary somewhat in sensitivity and it may prove necessary to either further dilute or to prepare higher concentrations of the working standards. Injection volumes may be varied from 5 to 25 µl.

V. SAMPLE COLLECTION:

The sampling schedule is a most important portion of this total project if meaningful data are to be obtained from a study of the urinary metabolites of the OP pesticides. If any type of surveillance or monitoring program is to be implemented, there must be a highly coordinated relationship between the chemist performing the analysis and the individual who plans the sampling schedules. It is strongly recommended that both individuals obtain copies of references 3, 4, and 5 as background material, and, if possible, discuss the proposed project with the senior authors of the publications cited.

This method, as a tool for determining the exposure index of the subject individual sampled, is considerably more sensitive to low levels of OP exposure than the ChE method given in Section 6,A,(3),(a) which measures the depression in blood cholinesterase.

In deciding on a sampling schedule, the time of day of taking the urine sample should be coordinated to the donor's working schedule since the urinary levels of alkylphosphate metabolites will vary with the time of sampling and the type of OP pesticide under study. Generally, the highest concentration of urinary metabolites is found from four to eight hours after the time of exposure. As a general rule, the best time to collect a urine sample is at the end of the work day.

VI. SAMPLE PREPARATION AND EXTRACTION:

1. Store urine in a freezer until ready for analysis. When the urine sample is thawed, mix well, centrifuge, and discard solids.

2. Pipet 1 ml of urine into a 13 ml centrifuge tube.

   NOTE: At this point, a sample of control urine from an unexposed donor should be started and carried through the entire procedure. The donor should be an individual known to have no contact with OP pesticides for at least a week.

3. Add 10 ml of acetone to precipitate some interfering compounds, including a large portion of the inorganic phosphate.

   NOTES:

   a. One of the major factors influencing the recovery of metabolites from urine was found to be the urine:acetone ratio used to precipitate the interfering compounds. In order to determine the optimum ratio, several urine: acetone ratios were investigated for each metabolite. One ml of urine was used throughout the experiment, and the volume of acetone was varied from 3 to 10 ml. As shown in Table 2, no single ratio was optimum for all alkyl phosphates and phosphonates. The lower amounts of acetone seem to favor some compounds, while the higher amounts seem to be best for others. When a specific alkyl phosphate is being sought, the urine:acetone ratio which gives the best recovery for that compound should be used. However, for a general screening method, a compromise ratio must be selected. The 1:10 ratio was selected for general use and gave cleaner chromatograms than the other ratios that were tried.

   b. The pH of urine-acetone mixture was investigated and found to have little effect on the recovery of the metabolites. The time required for complete derivatization was also checked, and it was found that, while most of the reaction was complete within one hour, increased yields could be obtained with overnight waiting periods.

4. Mix well with the Vortex mixer and centrifuge.

5. Prepare an ion exchange column as follows:

   a. Weigh one gram of ion exchange resin and slurry in 0.1 M HCl.

   b. Add the slurry to a 5 ml disposable pipet which has a plug of glass wool in the tip.

   c. Rinse the column with 5 ml of 0.1 M HCl followed by ca 50 ml of distilled water.

6. Transfer the supernatant from the centrifuge tube to the column using a disposable pipet, being careful to avoid any particles of residue.

7. Rinse the residue in the tube with 2 ml of acetone, centrifuge, and again transfer the supernatant to the column.

8. Allow the column to drain as much as possible.

9. Using a rubber suction bulb, blow the resin out of the column into a culture tube. Rinse the empty column with ca 1 ml of acetone and add to the culture tube.

10. To remove the metabolites from the resin, pipet 0.05 ml of 6 N HCl into the culture tube. Allow to stand one hour with occasional mixing of the resin.

VII. ALKYLATION:

1. After one hour, slowly add diazopentane reagent to the resin in the culture tube until a yellow color persists in the supernatant.

   NOTES:

   a. A large excess of diazopentane must be avoided since it is a major source of background interference.

   b. Be sure to do this work in a high draft hood.

2. Allow the reaction to proceed one hour, with occasional mixing.

   NOTE: Add more diazopentane reagent if at any time during this period the yellow color disappears.

3. With a disposable pipet, transfer the supernatant to a 13 ml graduated centrifuge tube, and wash the resin with small portions of acetone until a total volume of 10 ml is obtained.

4. Inject this solution into the gas chromatograph and compare to standards. If the level of alkyl phosphate metabolites appears too low for quantification, concentrate the sample to ca 0.2 ml under a nitrogen stream and chromatograph through silica gel. This procedure is used to remove interferences and quantify levels as low as 0.01 ppm. Alkyl phosphonate metabolites cannot be separated from interfering compounds by silica gel chromatography.

VIII. SILICA GEL COLUMN CHROMATOGRAPHY:

1. Prepare silica gel as follows: Partially deactivate 10 g of silica gel by shaking 2 hours with 2.0 ml distilled water. Transfer 2.4 g to a size 23 chromatographic column with a small wad of glass wool at the bottom. Top the column with ca 2 g of anhydrous sodium sulfate and prewash with 10 ml of hexane.

   (See Note on next page)

NOTE: Because elution patterns may vary from one laboratory to another depending on the temperature and relative humidity, each laboratory should establish an elution pattern of standards and spiked control urine samples under local conditions before analysis of samples.

2. Add 3 ml of hexane to the 13 ml centrifuge tube (Step 3 in Subsection VII), and transfer the sample to the column with a disposable pipet. Rinse the tube with two 2 ml portions of hexane and add to the column with the same pipet. Discard all eluate to this point.

3. Place a 25 ml concentrator tube under the column and add 15 ml of methylene chloride to the column. This is Fraction I, which contains DMTP and DETP.

4. Add 15 ml of acetone-methylene chloride (1:99 v/v) and discard this eluate. This eluate contains most of the triamyl phosphate, the thiolate derivatives, and other interfering substances.

5. Place another 25 ml concentrator tube under the column and add 20 ml of acetone-methylene chloride (3:97 v/v). This is Fraction II, which contains DMP and DEP.

IX. GAS CHROMATOGRAPHY:

1. For alkyl phosphates, the operating conditions of the gas chromatograph are:

Column temperature	140°C
Injection block temperature	200°C
Detector temperature	175°C
Nitrogen (carrier) flow rate	40 ml/minute
Hydrogen flow rate	50-60 ml/minute
Air flow rate	80-90 ml/minute

For the phosphonates, raise the column oven temperature to 160°C.

NOTES:

a. The temperature of the transfer block and the switching valve therein on the MT-222 chromatograph, which cannot be adjusted independently, ran ca 15° higher than the inlet on the instrument used. No oxygen is used with the model FPD employed with this procedure.

b. If an MT-220 chromatograph is used, transfer line and switching valve temperatures of 200°C are recommended. Since at least two different models of the FPD detector are in use, each laboratory should set the conditions of their FPD in accordance with the manufacturer's instructions.

2. Inject 5-25 μl of the 10 ml eluate from the ion exchange resin or Fractions I and II from the silica gel column if cleanup was carried out. Also, inject appropriate volumes of working standards (Subsection IV, 10) for comparison with samples.

3. Dilute or concentrate the sample depending on the chromatographic response from these initial injections.

4. Correct the amounts of metabolites found by subtracting the heights of interfering peaks found in the chromatogram of the control urine sample.

5. Correct results further based on recoveries from spiked control urine samples carried through the procedure.

6. Peak heights obtained in the phosphorus mode are used for comparison of working standards, samples, controls, and spikes (see Subsection XI).

NOTES:

a. DMTP and DETP isomerize upon alkylation, producing thionates and thiolates (Table 1). Quantification is based on the respective amyl thiolate derivatives because of greater interferences with the thionate peaks.

b. Any small amount of inorganic phosphate not precipitated by acetone or removed by silica gel column cleanup elutes from the GC column as triamyl phosphate with a late elution time ($R_T > 20$ minutes). An injection schedule can be adopted such that several sample injections are made in close succession, timed so that all peaks to be measured elute between the groups of interfering triamyl phosphate peaks.

See Figure 1 for chromatograms of amyl derivatives of alkyl phosphates and Figure 2 for chromatograms of phosphonate samples. In both cases, no cleanup step was carried out.

7. Do not inject Silyl 8 on a column directly connected to the FPD. If the column needs reconditioning, use the switching valve to vent the column effluent during the conditioning period.

NOTES:

a. After extended use of the gas chromatographic system, extraneous peaks may appear, chiefly from the accumulation of underivatized compounds on the column. Since the appearance of the extraneous peaks is not obvious, it is recommended that the operator inject about 1 μl of diazopentane solution periodically (2 weeks). If underivatized compounds show up as peaks following the diazopentane injection, recondition the column.

b. Quantification is based on the amyl derivatives DMAP, DEAP, DMAPTh, and DEAPTh. Some inorganic phosphate is extracted and converted to triamyl phosphate.

c. Confirmation and Specificity.

   (1) The ability to interchange the sulfur and phosphorus filters in the single detector, or the use of the base assembly for dual phototube operation with both filters (7), greatly enhances the specificity of this method. Suspected thiophosphate can be confirmed using the sulfur filter by increasing the concentration of the compound injected into the gas chromatograph by a factor of 5 to 10, if interference in the sulfur mode from urinary components is not excessive.

   (2) Confirmation of any particular compound can be accomplished by preparing the hexyl derivative. The method described for diazopentane is followed except that N-hexyl-N'-nitro-N-nitrosoguandine is used as the diazoalkane precursor.

   (3) Further confirmation is achieved using the silica gel column when the sulfur-containing derivatives are eluted in the methylene chloride fraction and the non-sulfur derivatives are eluted in the acetone-methylene chloride (3:97 v/v) fraction.

X. MISCELLANEOUS NOTES:

1. This method has been applied to media other than urine. Alkyl phosphates have been determined in whole blood, serum, stomach contents, and in liver from poison cases. Whole blood or serum is mixed with 10 parts of acetone, and analysis is continued as in Subsection VI,4. Tissue and other solid samples are first ground with equal parts of water (i.e., 0.5 ml water to 0.5 g sample). Ten ml of acetone is added for every one gram of slurry, and analysis is continued as in Subsection VI,4.

2. Since a minimum of 8 to 10 samples per day can be analyzed, the procedure is useful in a monitoring program or in clinical laboratories where the rapid classification of poisoning agents is essential.

3. DMP and DEP are the two most prevalent compounds detected. Whenever DETP shows up, DEP will invariably be present, and when DMTP shows up, DMP will invariably be present.

4. In a high exposure situation or a poisoning case resulting from malathion, DMP, DMTP, and DMDTP (O,O-dimethyl phosphorodithioate) may all be found. The latter compound is also alkylated by diazopentane, and the derivative will elute in Fraction I from the silica gel column. Its retention time on the GLC column will be between the derivatives of DMP and DEP.

5. Two recently published papers are related to this procedure:

   a. Blair, D., and Roderick, H. R., J. Agr. Food Chem. 24, 1221 (1976).

   b. Daughton, C. G., Crosby, D. G., Garnas, R. L., and Hsieh, D. P. H., J. Agr. Food Chem. 24, 236 (1976).

   The first paper describes the use of a cation exchange resin for isolation of urinary DMP, but the method is applicable only to DMP in the absence of other alkyl phosphates. Methyl derivatives are utilized which are highly volatile and difficult to resolve by GLC.

   The procedure in the second paper employs XAD-4 adsorbent to remove alkyl phosphates from aqueous media. A partitioning between water and ethyl acetate is included, which results in low recovery (ca 50%) of DMP. Diazomethane is again used for derivatization, leading to poor gas chromatographic resolution.

6. Table 3 shows recovery data obtained from spiked urine samples without silica gel cleanup. The recovery data are based on a 1:10 urine:acetone ratio for the alkyl phosphates, and on a 1:5 ratio for the phosphonates. The low recovery of *O*,*O*-dimethyl *O*-amyl phosphorothionate, DMTP, is due to an interfering peak that seems to come from the diazopentane reagent. However, since the other isomer, *O*,*O*-dimethyl *S*-amyl phosphorothiolate, DMPTh, can be quantified, the original level of *O*,*O*-dimethyl phosphorothioate can be determined (3).

7. Of the compounds investigated, only *O*-methyl phenylphosphonothioate, MPTPn, could not be quantitatively determined owing to an interfering peak that could not be resolved by GLC or removed by silica gel cleanup. The interference, which comes from the urine, can be reduced enough to allow qualitative determinations by using a 1:10 urine:acetone ratio.

8. The recovery of the metabolites varied from one urine sample to the next. Morning and afternoon urine samples were obtained from five donors to check the variability of the method. These samples were spiked with alkyl phosphates at 0.5 and 1.0 ppm. As shown in Table 3, the variation, which was possibly due to the variation in the salt content of different urines, was a little more than desirable.

9. The variability of urine is also a major factor influencing the limit of detectability, which is below 0.1 ppm for the alkyl phosphates. When the level of interference is low, urine samples may be evaporated to 1 ml for detection of very low levels of metabolites. If lower limits of detection are required, or if the interference level is too high, the sample must be carried through silica gel fractionation. The limit of detectability for the phosphonates ranged from 0.04 ppm for the *O*-methyl phenylphosphonic acid, MPPn, to 0.15 ppm for phenylphosphonic acid, PPn. Cleanup was found to be of little help with the phosphonates because the interference is not removed by silica gel.

10. The peak that interferes with the determination of MPTPn can be seen in the control urine chromatogram in Figure 1. The control urine chromatogram in Figure 2 shows the peak that interferes with the determination of DMP. However, the level of interference was generally low enough to allow quantification of levels above 0.1 ppm. With a highly efficient 5% OV-210 column (5000 theoretical plates), it is possible to obtain baseline separation of the alkyl phosphates. With less efficient GLC columns, the silica gel fractionation is required to separate the phosphate derivatives.

XI. ANALYTICAL QUALITY CONTROL:

Because the method is complex, routine analyses must be validated by conducting simultaneous analyses of spiked SPRM's (spiked reference materials). For occasional routine analyses, one SPRM should be analyzed with the unknown and in exactly the same manner. When large numbers of samples are analyzed, run at least one SPRM for every nine samples.

Because instability of a spiked urine sample precludes advance preparation of a large sample of SPRM for periodic analysis, prepare each SPRM as needed. The following procedure is suggested:

1. Prepare aqueous standard solution mixtures at 1.0 and 0.1 ppm containing each of the following compounds: DMP, DEP, DMTP, and DEPT. Use the spiking solution described in Subsections IV,10,a. and b. to prepare these solutions or proceed as follows: Weigh 10 mg of each into a one L volumetric flask, making to volume with water and shaking thoroughly. Dilute 10 ml of this solution to 100 ml. This will be STANDARD MIXTURE A. Transfer 10 ml of Mixture A to a 100 ml volumetric flask and make to volume with distilled water. This will be STANDARD MIXTURE B. Mixtures A and B will have the respective concentrations of 1.0 and 0.10 ppm.

2. Divide both mixtures into several screw cap test tubes or vials and freeze immediately. Do not fill the tubes over half full, and lay on side during freezing to reduce probability of cracking the glass.

3. When ready to conduct an SPRM analysis, thaw one tube (of each concentration) and draw a 0.1 ml aliquot to spike 1.0 ml of control urine (from an unexposed donor) contained in a 15 ml centrifuge tube.

   NOTE: If previous experience indicates that one of the two concentrations (0.1 or 1.0 ppm) will closely match the expected concentration in the unknown, only one concentration may be needed. Without such knowledge, prepare both concentrations.

4. In another centrifuge tube carry along a 1.0 ml unspiked urine sample from the same control donor.

5. Into another tube pipet 0.1 ml of the standard alone.

6. Proceed with the analysis of the two urine samples (spiked and control) as described in the procedure above.

7. To the tube containing the standard alone, add one drop of 6 N HCl, 1.0 ml of methanol and 2.0 ml of diazopentane, or a sufficient volume to give a persistent orange color. Mix well and allow to stand one hour (see Section IV,10,c).

8. Calculate recoveries by comparing the chromatographic data of the spiked samples with those of the corresponding standard.

TABLE 1. A LIST OF STANDARD METABOLITES AND THEIR DERIVATIVES**

Standard	Abbre-viation	Derivative(s)
O,O-dimethyl phosphate Na$^+$	DMP	O,O-dimethyl O-amyl phosphate
O,O-diethyl phosphoric acid	DEP	O,O-diethyl O-amyl phosphate
O,O-dimethyl phosphorothioate K$^+$ *	DMTP	O,O-dimethyl O-amyl phosphorothionate
	DMPTh	O,O-dimethyl S-amyl phosphorothiolate
O,O-diethyl phosphorothioate K$^+$ *	DETP	O,O-diethyl O-amyl phosphorothionate
	DEPTh	O,O-diethyl S-amyl phosphorothiolate
O-methyl phenylphosphonic acid	MPPn	O-methyl O-amyl phenylphosphonate
O-methyl phenyl phosphonothioate *	MPTPn	O-methyl O-amyl phenylphosphonothionate
	MPPnTh	O-methyl S-amyl phenylphosphonothiolate
phenylphosphonic acid	PPn	O,O-diamyl phenylphosphonate

* Compound forms two derivatives

** Phosphonates are from leptophos

TABLE 2. COMPARISON OF PERCENTAGE RECOVERY WITH VARIOUS URINE:ACETONE RATIOS

	Avg Recovery[a] With Ratios of		
	1:3	1:5	1:10
DMTP	b	90 (2)	39 (20)
DETP	b	81 (2)	77 (20)
DMP	b	85 (3)	85 (20)
DEP	b	65 (3)	105 (20)
DMPTh	b	Not analyzed	95 (20)
DEPTh	b	Not analyzed	87 (20)
MPTPn	Interference	Interference	74 (2)
MPPn	92 (6)	92 (6)	44 (2)
PPn	71 (6)	82 (6)	79 (2)

[a] Figure in parentheses represents the number of determinations

[b] Background too high to be useful

TABLE 3. RECOVERY, VARIABILITY, AND DETECTION LIMITS OF STANDARD DERIVATIVES

Compound	% Recovered[a] When Spiked With			Limit of Detection Without Cleanup (ppm)
	0.1 ppm	0.5 ppm	1.0 ppm	
DMTP	51	36 ± 14	42 ± 13	0.1
DETP	87	77 ± 10	76 ± 11	0.1
DMP	74	85 ± 20	85 ± 15	0.05
DEP	97	106 ± 15	105 ± 13	0.05
DMPTh	--	97 ± 23	93 ± 8	0.1
DEPTh	--	87 ± 9	87 ± 7	0.1
MPTPn	--	--	74	0.15
MPPn	97 ± 7	95 ± 14	90 ± 8	0.04
PPn	87 ± 11	86 ± 18	79 ± 22	0.07

[a] ± Standard deviation

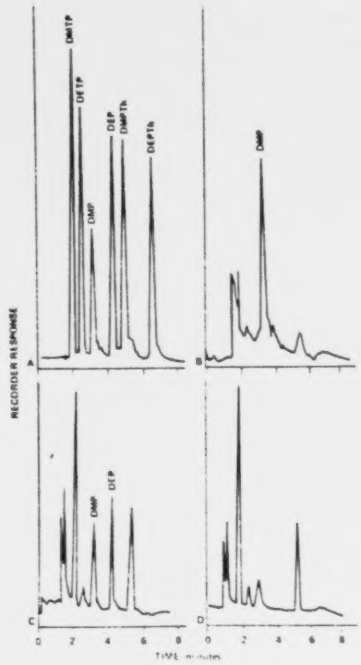


Figure 1.

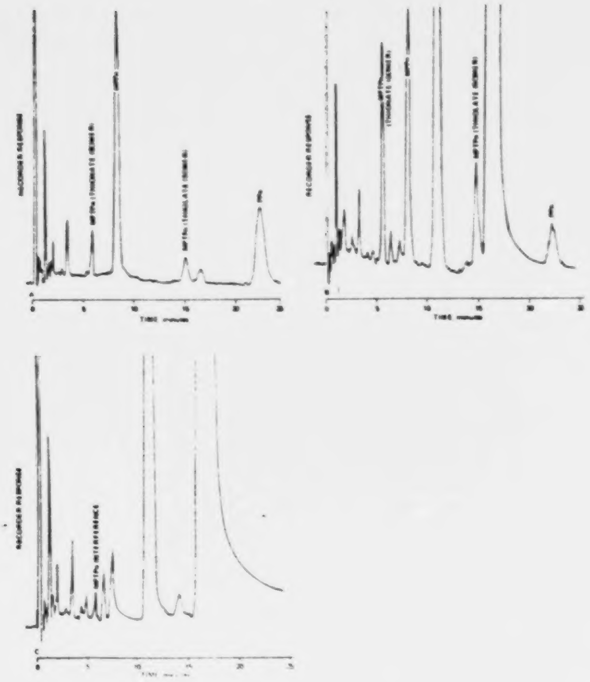


Figure 2.

Figure 1. Chromatograms of alkyl phosphates injected into a gas chromatograph without cleanup. A. Alkyl phosphate standards, 0.5 ng each. B. Urine extract from dichrotophos poison case. C. Extract of human urine spiked with 0.5 ppm of DMP and DEP. D. Control human urine.

Figure 2. Chromatograms of phosphonate samples injected into a gas chromatograph without cleanup. A. Phenylphosphonate standards, 0.5 ng each. B. Extract of urine from rats fed leptophos at 0.01 LD_{50}. Control rat urine.

DETERMINATION OF PARA-NITROPHENOL (PNP) IN URINE

I. INTRODUCTION:

Urinary PNP, the phenolic metabolite of parathion, methyl parathion, and EPN has been measured for years as an indicator of exposure to these organophosphorus pesticides. The Elliott spectrophotometric method is only semi-specific, requires a minimum of 10 μg of PNP, possesses marginal accuracy at low levels and is somewhat lengthy. The following gas chromatographic method yields acceptable analytical results at 50 ppb level and requires less than two hours for analysis. This method, in fact, deviates little from the Elliott method except for an added cleanup step and the determinative procedure.

REFERENCES:

1. Elliott, J. W., K. C. Walter, A. E. Penick, and W. F. Durham (1960). "A Sensitive Procedure for Urinary para-Nitrophenol Determination as a Measure of Exposure to Parathion." J. Agr. Food Chem. 8, 111.
2. Cranmer, M. F. (1970), "Determination of p-Nitrophenol in Human Urine." Bull. Environ. Contamin. and Toxicol., Vol. 5, No. 4, 329-332.

II. PRINCIPLES:

A small volume of urine is hydrolyzed with hydrochloric acid to free the PNP from the bound or adsorbed state. The hydrolyzed urine is made alkaline and extracted with benzene-ether to minimize co-extraction of interferences in the subsequent determinative extraction. The urine is then re-acidified and extracted with benzene-ether. The extract is dried, a suitable aliquot removed and the PNP converted on the column to the less polar and more volatile trimethylsilyl ether during the gas chromatographic determinative step.

III. APPARATUS:

1. Gas chromatograph with electron capture detector fitted with a column of 1.5% OV-17/1.95% QF-1 prescribed in the program.

2. Centrifuge tubes (distill. recvr.), grad., 12 ml, with Ŧ 14/20 outer joint, Kontes, #288250.

3. Stoppers, glass. flathead, Ŧ, 14/20, Kontes #850550.

4. Reflux condensers, equipped for water cooling with lower Ŧ inner joint the same size as test tubes. Kontes #282200.

5. Pipets, Mohr, 0.5 ml, grad. in 0.01 ml, Kimble #37023 or the equivalent.

6. Pipets, Mohr, 3 ml, grad. in 0.05 ml, Kimble #37023 or the equivalent.

7. Pipets, Mohr, 10 ml, grad. in 0.1 ml, Kimble #37033 or the equivalent.

8. Pipets, Transfer, 1 ml, Kimble #37000 or the equivalent.

9. Block tube heater, "Dri-Thermolyne," constant temp. 100°C, or comparable block heater with holes of appropriate size to accommodate centr. tubes.

10. Vials, glass, with screw caps, 15 x 45 mm, 1 dram, Kimble #60910.

11. Cap liners, Teflon, size 13, Arthur H. Thomas Co. #2849-D4.

IV. REAGENTS:

1. Hydrochloric acid, conc., A. R. grade.

2. Sodium hydroxide, A. R. grade, aqueous solutions of 0.1 N and 20%.

3. Benzene, pesticide quality.

4. Diethyl ether, pesticide quality.

5. Benzene-ether mixture - 80:20 (v/v).

6. Sodium sulfate, A. R. grade, anhydrous, granular.

7. Hexane, pesticide quality.

8. Hexamethyldisilizane reagent, 20% in hexane.

9. Hexamethyldisilizane reagent, 10% in hexane.

10. p-Nitrophenol standard solution of appropriate concentration range in hexamethyldisilizane - hexane solution (10:90 v/v). PNP standard-Eastman stock number EK192. The suggested concentration range for the working standard is 5 to 25 pg/µl.

V. HYDROLYSIS, EXTRACTION AND CLEANUP

1. With a 3 ml Mohr pipet, transfer 2.7 ml of urine into a 12 ml glass stoppered cent. tube and attach tube to a stoppered water cooled condenser.

   NOTE: At this point, a reagent blank consisting of 2.7 ml of distilled water and a control sample of 2.7 ml of urine from an unexposed donor should be carried through the entire procedure along with the suspect sample(s).

2. Add with a grad. 0.5 ml Mohr pipet, exactly 0.30 ml of conc. HCl and reflux the mixture for 1 hour with tube inserted in "Dri-Thermolyne" block heater.

   NOTE: During the refluxing period, the condenser must be stoppered and cooled by water circulation.

3. Remove assembly from heat and rinse down condenser with 2 ml of 0.1 N NaOH. Cool tube and adjust to a pH of 11 or higher with 0.4 ml of 20% NaOH solution.

4. Add 5 ml of the 80:20 benzene-ether reagent, stopper tube, and shake vigorously 1 minute.

5. Remove as much as possible of the benzene-ether (upper) layer with a disposable pipet and repeat extraction one more time with another 5 ml of the benzene-ether, discarding the benzene-ether extract from both extractions.

6. Reacidify the urine to pH 2 or lower with ca 0.2 ml conc. HCl, add 5.4 ml of the benzene-ether solvent, stopper tube, and shake vigorously 1 min.

7. Using a disposable pipet, carefully transfer as much of the solvent (upper) layer as possible into a second centrifuge tube, taking care that no aqueous phase is included.

8. Add 0.5 grams anhydrous Na_2SO_4, stopper tube, and shake vigorously 1 min. to remove traces of moisture from the solvent extract.

9. Transfer 1 ml of the dried benzene-ether extract to a 1 dram glass vial with a Teflon lined screw cap, then add 1 ml of 20% hexamethyldisilizane in hexane. Cap vial and shake vigorously 1 min.

VI. GAS CHROMATOGRAPHY:

Before injecting the sample extract, pre-condition the column with several repetitive injections of the PNP/HMDS-hexane standard (Subsection IV,10). This serves the dual purpose of (1) providing a quantitative standard peak, and (2) conditioning the column prior to sample injection.

NOTES:

1. During the course of sample injections the column must be monitored to determine whether all the PNP injected is being converted on-column to PNP-TMS. This is done by injecting HMDS-hexane (10:90 v/v) without PNP. If a PNP peak is produced, it is indicated that the column has adsorbed PNP and requires further conditioning with HMDS.

2. The author reported recoveries greater than 90% for PNP levels down to 25 ppb in urine.

☆ CHOLINESTERASE ACTIVITY IN BLOOD

I. INTRODUCTION:

The cholinesterase enzyme in blood is inhibited in varying degrees by organophosphate pesticides and is loosely correlated with the decrease of acetylcholinesterase activity in the nervous system which in turn is accompanied by an increase in the concentration of acetylcholine. Therefore, a scheme for measuring the level of activity of the cholinesterase is one means of establishing possible exposure.

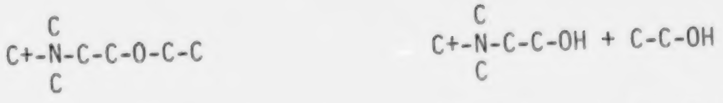
The technique of continuous titration of the acetic acid released from acetylcholine by the enzyme cholinesterase overcomes many of the undesirable features of other methods. This method does not utilize buffers, is temperature and atmospherically controlled, and has easily calculated units. In addition, the substrate and enzyme concentrations can be adjusted and maintained at levels which allow optimal enzyme activity.

REFERENCES:

1. Michel, H. O. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. J. Lab Clin. Med. 34; 1964 (1949).
2. Nabb, D. P. and F. Whitfield. Determination of cholinesterase by an automated pH-stat method. Arch. Environmental Health 15:147 (1967).
3. Pearson, J. R. and G. F. Walker. Conversion of acetylcholinesterase activity values from the Michel to the pH-stat scales. Arch. Environmental Health, 16:809 (1968).

II. PRINCIPLES:

The whole blood sample is separated into the plasma and RBC (red blood cells). Each fraction is placed in the reaction vessel of a pH-stat and mixed with an excess of acetylcholine iodide. The cholinesterase present in the blood fraction reacts with the AChI releasing acetic acid as illustrated in the following:



$$C{+}\text{-}\overset{C}{\underset{C}{N}}\text{-}C\text{-}C\text{-}O\text{-}C\text{-}C \qquad C{+}\text{-}\overset{C}{\underset{C}{N}}\text{-}C\text{-}C\text{-}OH + C\text{-}C\text{-}OH$$

Acetylcholine
(ACh)

Choline

Acetic Acid
HA

A standardized solution of dilute NaOH is used as the titrant for the released HA. The automatic titrator records the amount of titrant delivered to the reaction in a given time period. A feedback signal from the pH measuring electrodes controls the titrant delivery rate. A high ChE activity produces a large hydrogen ion release in a fixed time period, calling for a faster titrant delivery rate than will a lower ChE activity.

III. EQUIPMENT:

1. *pH-stat, recording, complete with micro glass reference combination electrode, thermistor temperature sensing element, a buret assembly for delivery of 0.5 and 2.5 ml, reaction vessels.
2. Vortex mixer.
3. Centrifuge capable of spin velocity of 2000 rpm.
4. Aspirator for connection to suction pipet.
5. Pipet, volumetric, 2 ml.
6. Pipets, measuring, 0.2, 0.5 and 5.0 ml, Corning 7064 or the equivalent.
7. Centrifuge tubes, 5 ml, glass stoppered, Corning 8061 or the equivalent.

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*Manufacturers of equip. applicable for automatic pH titration are:

Burkland Scientific, 919 North Michigan Avenue, Chicago, Illinois.
Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania.
Joseph Kaye, 737 Concord Avenue, Cambridge, Massachusetts.
Metrohm-Brinkmann, Cantiague Road, Westbury, New York.
Precision Scientific, 3737 West Cortland, Chicago, Illinois.
Radiometer-London, 811 Sharon Drive, Westlake, Ohio.
E. H. Sargent, 4647 West Foster Avenue, Chicago, Illinois.

IV. REAGENTS:

1. Sodium chloride, reag. grade.

   Prepare 0.9% solution by dissolving 9.0 grams and diluting to a liter with dist. water.

2. Potassium acid phthalate (P.A.T.) - analytical primary standard, available from the National Bureau of Standards, Washington, D.C.

   Standard Solution - Dry standard P.A.T. in 105°C oven at least 4 hours before use. Store dried salt in desiccator. Weigh exactly 0.20423 grams and transfer to a 1-liter vol. flask, dissolving and making to volume in freshly boiled dist. water.

3. Titrant standard solutions prepared from sodium hydroxide, reag. grade, Fisher S-318 or the equivalent, 98.7% NaOH.

   a. Stock solution (1.0 N NaOH): Weigh 4.053 grams NaOH, dissolve in freshly boiled dist. water, cool, and dilute to 100 ml. Store in Pyrex bottle with neoprene rubber stopper.

   b. Working solution (0.01 N NaOH): Pipet 1.0 ml of the 1.0 N solution into a 100-ml vol. flask and make to volume with freshly boiled dist. water. Standardize using potassium acid phthalate as the primary reference.

4. Standardization of titrant working solution.

   This solution should be restandardized each time that a series of samples is run. Triplicate standardizations are run to obtain an average, with the deviation between replicates no greater than 2 RU (recorder units). It is not mandatory that the exact normality is known, but the value should fall in the range of 0.0095 to 0.0105.

   The standard solution of P.A.T. contains 0.001 meq/ml. The neutralization of each ml of the P.A.T. solution will require 0.001 meq of NaOH. The NaOh working solution contains 0.01 meq/ml. Therefore, 0.1 ml of the working titrant ≅ 1.00 ml of the P.A.T. solution.

   a. With a vol. pipet, transfer 2 ml of the standard P.A.T. solution into a clean titration vessel and normalize instrument operating temperature to 37°C.

b. Set titrant delivery to appropriate position for 2.5 ml delivered full scale, set pH to 7.0, position pen at zero, and turn on function switch.

c. The end point is reached when pen levels off at a certain RU level. Record this level and compute the titrant normality as follows:

$$N = \frac{2.0 \text{ ml} \times 0.001 \text{ meq/ml}}{\text{RU} \times 0.005 \text{ ml/RU}} = \frac{0.4}{\text{RU}} \text{ meq/ml}$$

NOTE: Total syringe delivery in the assumed system is 0.50 ml, indicated by 100 RU, or 100% of full syringe travel. It is not convenient to use the entire syringe volume for a titrant standardization or assay. For other syringe delivery capacities, such as 0.25 or 1.0 ml, adjust the RU value accordingly.

5. Acetylcholine iodide is available from Calbiochem Company, P. O. Box 54282, Los Angeles, California 90054.

Substrate solution - Weigh 0.7510 grams of AChI into a 25-ml vol. flask. Dissolve and make to volume at room temperature. Store in an amber bottle in the refrigerator and hold no longer than 2 weeks.

NOTE: Weigh sample without delay since all salts of acetylcholine are hygroscopic and weight will change rapidly.

V. SAMPLE HANDLING AND PREPARATION:

The sample preparation and analysis of blood should be carried out as soon as possible after drawing sample. If a few hours delay is unavoidable, keep samples refrigerated. If the delay will be overnight or longer, blood should be centrifuged and plasma separated from RB cells and the latter taken through the following steps (a through f) before storage.

a. Place blood tube in centrifuge and spin for 20 minutes at 2000 rpm.

b. Pipet plasma into clean tubes for storage.

NOTE: If a part of this sample will eventually be analyzed by EC GLC, the tube cap should be ground glass or screw cap with Teflon or foil liner.

c. Using vacuum pipet, remove and discard fluffy layer.

d. Resuspend red cell mass in an equal volume of 0.9% NaCl solution. This is done by gently inverting tube.

e. Centrifuge again for 10 minutes and remove supernatant NaCl colution by vacuum pipet.

f. Repeat steps d. and e. The RBC mass is now ready for assay.

VI. PREPARATION OF RBC HEMOLYSATE:

1. Pipet 1.8 ml of dist. water into a 5 ml conical centrifuge tube and then pipet in 0.2 ml of packed red blood cells.

   NOTE: Care must be taken to wipe the tip of the pipet with tissue while still retaining the full 0.2 ml contents. This may require some practice.

2. With a rubber bulb attached to the pipet, draw the hemolysate solution up into the bore of the pipet repeatedly until all adhering cells are washed into the water.

3. Stopper tube and mix on Vortex mixer about 30 seconds or until all cells have hemolyzed. The hemolysate so prepared may remain up to 20 minutes at room temperature before assaying. For longer periods, hold in refrigerator.

VII. CHOLINESTERASE ASSAY:

1. Calibrate instrument with reference buffers, following manufacturer's instructions. This is best done by using two buffers, one below and one slightly above pH 8. Normalize the instrument to a 37°C operating temperature.

2. Into a clean titration vessel pipet 4.2 ml of 0.9% NaCl solution and 0.15 ml of plasma, (or 4.2 ml of 0.9% NaCl and 0.50 ml of RBC).

3. Place titration vessel on instrument and be sure that instrument end point is set at pH 8.0. As the initial pH of sample and NaCl solution will nearly always be a little low, it is necessary to adjust the pH to 8.0 with the 0.01 N NaOH titrant.

4. Check to be sure that recorder and titrant delivery systems are set at zero.

5. Add substrate to mixture in titration vessel:

    a. If plasma assay, add 0.6 ml of acetylcholine iodide solution.

    b. If RBC assay, add 0.10 ml of AChI solution.

6. Start recorder and titrant flow and allow titration to proceed until recorder describes a line with constant slope, then mark off the start and end of a 3-minute period of titration. See Figure 1.

    NOTE: None of the line included should be nonlinear. It is good practice to allow titration to proceed for a minute or so before counting the RU.

VIII. SAMPLE CALCULATIONS:

Refer to Figure 1:

1. Standardization of titrant.

Average of three replicate titrations:

$$\begin{array}{r} 54.0 \text{ RU} \\ 54.0 \\ \underline{53.9} \\ 3\overline{)\,161.9} = 53.961 \text{ RU} \end{array}$$

From formula (1), $N = \frac{0.600}{53.961} = \frac{0.600}{53.961} = 0.011$ N

2. Calculation of ChE activity.

    A. Factor, from Table 1, for plasma ChE, based on 0.011 N titrant, is 0.1222. The observed RU value is multiplied by this factor:

Plasma 1: 46.5 x 0.1222 = 5.682 μM/min/ml (first replicate)
48.5 x 0.1222 = 5.927 μM/min/ml (second replicate)

Plasma 2: 30.5 x 0.1222 = 3.727 μM/min/ml (only one replicate shown)

    B. Alternative method, without using factors:

Plasma 1: $\frac{\text{RU x 0.005 ml/RU}}{\text{Minutes}}$ = ml/minute = Substituted: $\frac{46.5 \times 0.005}{3}$ = 0.0775 ml/min.

$$\text{ml/minute} \times \text{Normality} = \text{meq/minute} = 0.0775 \times 0.011 = 0.000853\ \text{meq/minute}$$

$$\text{meq/minute} \times 1000\ \mu\text{M/meq} = \mu\text{M/minute} = 0.000853 \times 1000\ \mu\text{M/meq} = 0.853\ \mu\text{M/minute}$$

$$\frac{\mu\text{M/minute}}{\text{sample volume}} = \mu\text{M/min/ml} = \frac{0.853}{0.15} = 5.687\ \mu\text{M/min/ml}$$

IX. MISCELLANEOUS NOTES:

1. Because of the variety of instruments available, operating instructions are not given for pH-stat equipment. Detailed instructions furnished by the manufacturer will govern operating details. A step-by-step outline of sample preparation, reagent preparation, and standardization, plus the scheme for performing calculations are outlined.

2. Heparin is preferable to other anticoagulants, because oxalate, citrate, and EDTA will sequester calcium and magnesium, which are required co-factors for ChE. In an emergency, these anticoagulants can be used, but ChE assay results are likely to be lower than if heparin were used.

3. Considering the low pipetting volumes, meticulous care must be taken to obtain reproducible aliquots. This is particularly true with packed red blood cells.

4. Centrifugation speeds and times should be consistent from one sample lot to the next. Formation of packed red cell mass after final saline wash is critical. Each successive red cell pack should be the same, otherwise differences in density will yield different results from sample to sample.

5. Be Gentle. Hemolysis of red cells in contact with plasma will liberate acetylcholinesterase into plasma, thus altering the true enzyme activity of the latter. Mix red cells with plasma or saline solution by gentle inversion of tubes, or by gentle stirring with glass rod or wooden applicator stick.

6. The approximate lower limits of normal ChE activity for human blood assayed by the present method are:

   2.0 μM/min/ml - Plasma
   8.0 μM/min/ml - Red Cells

An individual laboratory should, of course, establish its own normal ranges based on experience with the method.

7. Assay results obtained by the pH-stat method are expressed as:

   micromoles (acetic acid liberated)/minute/ml sample (either packed red cells or plasma). Abbreviated: μM/min/ml.

8. Standard sources of enzyme are available from Sigma and are useful in intralaboratory quality control.

FIGURE 1. Sample of strip chart of pH-stat assay of cholinesterase activity.

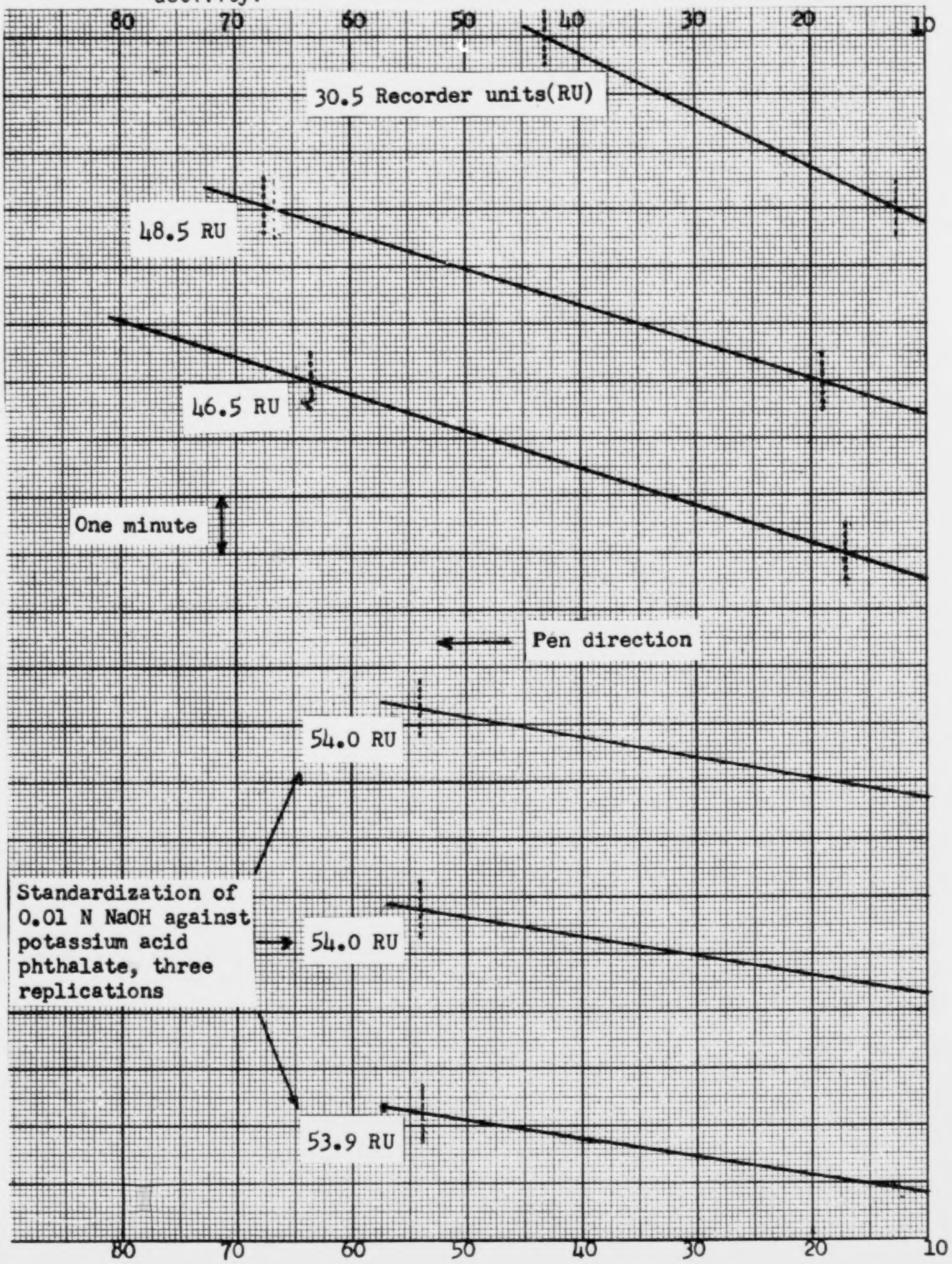

TABLE 1. FACTORS FOR THREE MINUTE TITRATIONS

Normality NaOH Solution	Factor	Plasma ChE Factor
0.	0.	0.
0095	3167	1055
0096	3200	1067
0097	3233	1078
0098	3267	1089
0099	3300	1100
0100	3333	1111
0101	3367	1122
0102	3400	1133
0103	3433	1144
0104	3467	1156
0105	3500	1167

These factors are derived as follows:

$$\text{Red cell factor} = \frac{\text{ml/min} \times \text{meq/ml} \times 1000\ \mu\text{M/meq}}{0.05}$$

$$\text{Plasma factor} = \frac{\text{ml/min} \times \text{meq/ml} \times 1000\ \mu\text{M/meq}}{0.15}$$

These factors are valid only if a 0.5-ml syringe is used for titrant delivery, so that the ml/min factor in the equation becomes $\frac{0.005}{3}$, or 0.00167 ml/min.

DETERMINATION OF 1-NAPHTHOL IN URINE

I. INTRODUCTION:

Humans exposed industrially to the insecticide carbaryl (1-naphthyl-N-methyl carbamate) excrete relatively large quantities of 1-naphthol, conjugated either as the sulfate or glucuronide. Quantitative determination of 1-naphthol in human urine has been generally accomplished using a colorimetric procedure. This method lacks both the sensitivity and specificity necessary for determining the relatively small amounts of 1-naphthol excreted in the urine of agricultural workers exposed to low levels of this insecticide.

The 1-naphthol resulting from the hydrolysis of carbaryl has been used as an indirect measure of the residue level of the parent insecticide on a variety of agricultural crops. Argauer has described a procedure for chloroacetylating phenols and 1-naphthol for subsequent detection by electron capture gas chromatography. In a recent publication, this procedure was utilized to determine a number of carbamate insecticides. However, it was indicated that further modification would be necessary if the method was to be extended to carbaryl.

The method described in this section utilizes the enhanced electron capture characteristics of the monochloroacetate derivative. This, coupled with a silica gel cleanup results in a method sensitivity down to 20 ppb of 1-naphthol.

REFERENCES:

1. Shafik, M. T., Sullivan, H. C., Enos, H. F., Bull of Environ. Contamin. & Toxic., Vol. 6, No. 1, 1971 pp 34-39.

II. PRINCIPLES:

A small sample of urine is subjected to acid hydrolysis. The 1-naphthol present is extracted in benzene and derivatized with chloroacetate anhydride solution. After silica gel cleanup, the resulting 1-naphthyl chloroacetate is quantitatively determined by EC,GLC, comparing sample peaks against peaks obtained from pure 1-naphthol standard, similarly derivatized.

III. APPARATUS:

1. Gas chromatograph equipped with EC detector and fitted with a 6' x 1/4 " o.d. column of 1.5% OV-17/1.95% QF-1. Operating parameters for the column are those prescribed in Section 4,A of this manual.

2. Chromatographic column (Chromaflex), size 22, Kontes #420100.

3. Evap. concentrator tubes grad., glass stoppered, 25 ml, $ 19/22, Kontes #570050.

4. Distilling column (condensor), 200 mm jacket, Kontes #286810, fitted with tight glass stopper at top.

5. Centrifuge capable of 2000 rpm that will accept metal shield, I. E. Company No. 367.

6. Volumetric flasks, 10 and 100 ml.

7. Pipets, Mohr, 0.2, 0.5 and 10 ml, Corning 7064 or the equivalent.

8. Pipets, transfer, 2, 3 and 5 ml, Corning 7100 or the equivalent.

9. Vortex-Genie mixer.

10. Disposable pipets, Pasteur, 9 inch.

11. Graduated conical centrifuge tubes, 15 ml, with $ glass stoppers, Corning 8084 or the equivalent.

12. Circulating water pump.

13. Boiling water or steam bath.

IV. SOLVENTS AND REAGENTS:

1. Benzene, pesticide quality.

2. Hexane, pesticide quality.

3. Pyridine, Spectro Grade, Eastman #13098.

4. Chloroacetic anhydride, Eastman #335 - prepare a 2% solution in benzene and hold no longer than one week.

NOTE: The chloroacetic anhydride must be dry and therefore should be stored in a desiccator as it is extremely hygroscopic.

5. Hydrochloric Acid, reag., conc.

6. Sodium sulfate, anhydrous, granular - preextract on a Soxhlet with benzene for about 50 discharge cycles, remove excess solvent and store in 130°C oven.

7. Sodium sulfate, 3% solution in dist. water. Use preextracted Na_2SO_4.

NOTE: Deionized or distilled water, preextracted with benzene, is used throughout the procedure.

8. Mixtures of benzene/hexane as follows: 20/80, 40/60 and 80/20.

9. 0.1 N and 1.0 N NaOH solutions.

10. Silica gel, Woelm, activity grade I, Waters Associates, Inc., DO NOT SUBSTITUTE.

11. Preparation of silica gel.

Dry adsorbent for 48 hours at 170°C and store in the same oven. On day of use, cool the silica gel in a desiccator and deactivate with 1.5% water in the following manner: Add the necessary volume of water to a 125-ml glass stoppered Erlenmeyer flask, rotating the flask to coat the sides with water. Add the weighed amount of silica gel, stopper, and mix until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hours with periodic shaking. Chromatographic columns are prepared just prior to use.

12. 1-Naphthol, Eastman #170 or Reference Standards Repository, EPA, Research Triangle Park, NC.

13. Preparation of standard solutions;

    a. Stock Standard I. Weigh 20 mg of 1-naphthol into a 100-ml vol. flask, dissolve and dilute to volume with benzene. This is the conc. stock of 200 ng/μl and may be held several months at -18°C.

b. Stock Standard II. With a 0.5-ml Mohr pipet, transfer 0.25 ml of stock standard I to a 50-ml vol. flask and make to volume with benzene. This intermediary stock standard of 1 ng/μl is used for preparation of working standards and sodium naphthoxide for recovery studies (See Misc. Note, 1, a).

c. Prepare reagent blank and working standards by transferring aliquots of stock standard II of 0, 0.1 and 0.5 ml to separate 25 ml evap. concentrator tubes. Dilute each to 5 ml with benzene.

   (1) Add 2 ml of 2% chloroacetic anhydride and 0.2 ml of pyridine to each tube. Stopper and mix vigorously on Vortex mixer for 2 minutes. Allow to stand 10 minutes.

   (2) Add 5 ml of dist. water, stopper and reagitate on Vortex for one minute.

   (3) Allow layers to separate and, with a disposable pipet, carefully remove and discard as much as possible of the lower (aqueous) layer.

   (4) Repeat water wash (Steps (2) and (3) above) twice more.

   (5) Place tubes in centrifuge, spin 5 minutes at 2,000 rpm and remove any final traces of water from bottom of tubes. Dilute to exactly 10 ml with benzene and mix thoroughly. The three tubes will contain concentrations of 1-naphthyl chloroacetate of 0, 10 and 50 pg/μl.

d. Prepare another intermediary stock standard (III) of derivatized 1-naphthol (1-naphthyl chloroacetate) by transferring 0.5 ml of stock standard I to a 25-ml evap. concentrator tube and dilute to 5 ml with benzene. Proceed with derivatization as described above in c, (1), (2), (3), (4) and (5) but instead of diluting to 10 ml as described in step (5), transfer extract through a glass funnel into a 100-ml vol. flask, rinsing tube with several portions of benzene and finally making to volume with benzene. From this derivatized stock of 1 ng/μl concentration of 1-naphthyl chloroacetate, dilutions may be made and used to check the derivatized working standards finalized in Step c, (5) above.

NOTE: The derivatized standards are relatively stable. Assuming no solvent losses from repeated opening of the storage bottles, it is probable that the standards could be held for 6 months, storing in the refrigerator when not in use.

V. HYDROLYSIS, EXTRACTION AND DERIVATIZATION:

When the following procedure is started there should be no interruption until the final derivatized extract is obtained in Step 10. It is highly desirable that a sample of control urine from an unexposed donor be carried along parallel with the sample(s) under test.

1. Pipet 5 ml of urine into a 25 ml evap. concentrator tube, add 1 ml of conc. HCl, stopper, and mix on the Vortex mixer 2 minutes.

2. Fit concentrator tube with a glass stoppered condenser (distilling column) and reflux mixture in a hot water or steam bath 90 minutes, cooling the condenser with circulating ice water.

   NOTE: The top of the condenser should be tightly stoppered.

3. After removal from the bath and cooling, wash down bore and condenser tip with 2 ml of 0.1 N NaOH followed by 3 ml of benzene.

4. Stopper and mix vigorously for 2 minutes on the Vortex mixer.

5. Place conc. tube in centrifuge and spin for 10 minutes at 2,000 rpm.

6. Carefully transfer as much of the benzene (upper) layer as possible to a clean 25 ml concentrator tube, using a disposable pipet fitted with a rubber bulb.

7. Add 3 ml more of benzene and repeat Steps 4, 5 and 6.

   NOTE: Extreme care should be taken to prevent water from being transferred as this would seriously affect derivatization efficiency.

8. Wash benzene extract with two 3 ml portions of 3% Na_2SO_4 solution, centrifuging and discarding each successive aqueous layer.

9. To the combined benzene extract add 2 ml of 2% chloroacetic anhydride solution and 0.1 ml of pyridine. Stopper tube, mix on Vortex mixer for 2 minutes, and allow to stand 10 minutes at room temperature.

10. Refer to and follow identically Steps (2), (3), (4), and (5) of Subsection IV, 13, C, but placing tubes of derivatized extract in a 40°C bath and evaporating to 0.5 ml under a dry nitrogen stream.

    NOTE: Under no circumstances should final volume be permitted to go below 0.3 ml.

VI. SILICA GEL FRACTIONATION:

1. Place a small wad of glass wool at the bottom of a Chromaflex column and add 1 gram of the partially deactivated silica gel. Top this with ca 1/2-inch of anhydrous, granular Na_2SO_4.

2. Prewash the column with 10 ml of hexane, discarding the eluate.

3. When the surface level of the nexane reaches a point on the column ca 2 cm from the top of the Na_2SO_4 transfer the concentrated benzene extract to the column with a disposable pipet and rinse tube with two portions of 0.5 ml of 20/80 benzene/hexane solvent applied with another disposable pipet, directing stream so as to wash down walls of tube. Follow this with 8.5 ml of 20/80 benzene/hexane solvent, discarding all eluates up to this point.

4. Place a 15 ml grad. conical centrifuge tube under column and add 10 ml of 60/40 benzene/hexane solvent, collecting this fraction which contains the 1-naphthyl chloroacetate derivative. Finally, adjust volume of extract to exactly 10 ml with benzene.

   NOTES:

   1. Elution patterns may vary from one laboratory to another depending on the temperature and relative humidity. This emphasizes the need for establishing an elution pattern of standards and spiked control urine samples under local conditions before attempting to analyze samples. The procedure for spiked control urine samples is as follows:

      a. In a 15-ml conical grad. centrifuge tube mix 2 ml of 1.0 N NaOH and 2 ml of the diluted, underivatized standard described in the NOTE in Step b, of Subsection IV, 13.

b. Stopper tube and mix 2 minutes on a Vortex mixer. Allow to stand 10 minutes and centrifuge 5 minutes at 2,000 rpm.

c. Pipet aliquots of 0.1, 0.25 and 0.5 from the aqueous layer (sodium naphthoxide solution) into separate 25 ml grad. evap. concentrator tubes.

d. From this point on, conduct the hydrolysis and derivatization as previously described in Subsection V, starting with Step 1, under Hydrolysis, Extraction and Derivatization, ending at Step 4 under Silica Gel Cleanup. The final extracts contained in the three 15-ml centrif. tubes should have concentrations of 10, 25 and 50 pg/μl of derivatized 1-naphthyl chloroacetate. Recovery data are obtained by chromatographing these extracts against the derivatized working standards.

2. At no time during the elution should the liquid level in the column be allowed to drop below the top surface of the Na_2SO_4 bed.

VII. GAS CHROMATOGRAPHY:

After adjusting operating parameters of the gas chromatograph to the values prescribed in Section 4,A of this manual, commence injections of derivatized sample and standard extracts. Assuming an average background current, it should be possible to quantify as little as 50 picograms of the derivatized 1-naphthol. Using the 1.5% OV-17/1.95% QF-1 column, the relative retention value for 1-naphthyl chloroacetate should be ca 0.92 with respect to aldrin.

VIII. MISCELLANEOUS NOTES:

1. Elution patterns may vary from one laboratory to another depending on the temperature and relative humidity. This emphasizes the need for establishing an elution pattern of standards and spiked control urine samples under local conditions before attempting to analyze samples. The procedure for spiked control urine samples is as follows:

a. In a 15-ml conical grad. centrifuge tube mix 2 ml of 1.0 N NaOH and 2 ml of the diluted, underivatized standard described in the NOTE in Step b, of Subsection IV, 13.

b. Stopper tube and mix 2 minutes on a Vortex mixer. Allow to stand 10 minutes and centrifuge 5 minutes at 2000 rpm.

c. Pipet aliquots of 0.1, 0.25 and 0.5 from the aqueous layer (sodium naphthoxide solution) into separate 25 ml grad. evap. concentrator tubes.

d. From this point on, conduct the hydrolysis and derivatization as previously described in Subsection V, starting with Step 1, under Hydrolysis, Extraction and Derivatization, ending at Step 4 under Silica Gel Cleanup. The final extracts contained in the three 15 ml centrif. tubes should have concentrations of 10, 25 and 50 pg/µl of derivatized 1-naphthyl chloroacetate. Recovery data are obtained by chromatographing these extracts against the derivatized working standards.

SAMPLING OF PESTICIDES IN AIR

I. INTRODUCTION:

Pesticides in air represent an important class of toxic pollutants, which may have chronic deleterious effects on human health and the ecological balance. In 1963, the President's Science Advisory Committee recommended that the air be continuously monitored for pesticides. The authority for such monitoring in the U. S. has been granted to the Environmental Protection Agency under the Clean Air Act as amended in December 1970, and the Federal Insecticide, Fungicide, and Rodenticide Act as amended in 1972.

Presently, there are only limited data concerning the contamination of the atmosphere by pesticides, especially in urban areas. Information relating to transport or ambient trends is even less available. Such information must be obtained before total air quality can be defined and before the threat of atmospheric pesticidal pollutants to the general populace and the ecosystem can be determined.

The determination of pesticides in the ambient air is a formidable task. There are hundreds of pesticides registered for use in the U. S., many of which are potential air pollutants. These pesticides may exist in air as vapors, aerosols, or adsorbed on suspended particulate matter; thus, their collection is complicated. Pesticides are usually present in air at levels far lower than those found in crop residues for which most methods of analysis are designed; hence, their detection is difficult. Metabolites and degradation products of pesticides, which are sometimes considerably more toxic than the parent pesticide, are, of course, at even lower atmospheric concentrations.

Most of the existing data concerning the nature and degree of contamination of the ambient atmosphere by pesticides was collected over the period from 1970 to 1972 by the EPA. The sampling method utilized was based on impingement in ethylene glycol, which was expensive and cumbersome to use and did not provide an adequate sample size to permit subnanogram per cubic meter detectabilities for most pesticides. Sections 8,B and 8,C in the 6/77 revision of this Manual were based on collection of samples in ethylene glycol. During the past several years, EPA has developed a high volume air sampler that is believed to better serve the needs for pesticide ambient air monitoring. This sampling device and others for indoor air sampling, crop re-entry monitoring, and workplace and personnel monitoring are described in this subsection, and analytical methodology for the pesticides collected in these samplers are detailed in Section 8,B.

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2. Sampling and Analysis of Airborne Pesticides, Lewis, R. G., in Air Pollution from Pesticides and Agricultural Processes, R. E. Lee, Jr., (Ed.), CRC Press, 1976, pp. 52-94.

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5. Sources of Emissions of Polychlorinated Biphenyls into the Ambient Atmosphere and Indoor Air, MacLeod, K. E., EPA-600/4-78-022, March, 1979. Analytical Chemistry Branch, ETD, HERL, Research Triangle Park, NC 27711.

II. AMBIENT AIR SAMPLING:

1. Descriptions of Ambient Air Samplers

For ambient (uncontaminated) air, sufficiently large samples must be taken to permit detection and measurement at ultratrace levels (pg/m^3 to ng/m^3). Such sampling should be performed over an entire diurnal cycle if results are to be representative of the average quantities of the substances normally present in the atmosphere.

a. The sampler developed by EPA has been referred to as the modified SURC sampler, since it is similar to a high volume pesticide air sampler designed for EPA by Syracuse University Research Corporation. The device uses a Hi-Vol pump and shelter, and draws air through a glass fiber filter (to collect particulate matter) and a solid sorbent cartridge (to trap vapors) at sampling rates up to 280 liters/minute. The sampler can be used with a wide variety of sorbents in a manner that permits their continual re-use. It is designed for low cost and simple operation. The sampler has been demonstrated to efficiently collect a number of organochlorine and organophosphate pesticides, and it is presently being evaluated for carbamates.

A standard aluminum Hi-Vol sampler housing is modified by replacing the sheet aluminum support plate with one that is 9 mm thick. A second support plate is added approximately 25 cm from the bottom of the sampler to lend strength. The top plate forms the support for the blower unit and pesticide collection module as well as the necessary plumbing.

All parts and how they are connected are shown in Figure 1. A variable power transformer is provided to adjust the vacuum pulled by changing the motor speed. This prolongs the life of the motor. The flow is measured by two devices: a Dickson recorder, which keeps a continuous record of the flow versus time, and a venturi (Barco Model BR-12402-08-31) - Magnehelic gauge (Dwyer Instruments Model 2100), used to set the flow rate of the sampler when in operation. The exhaust duct is required to stop recycling of the air.

b. PCBs have been collected on polyurethane foam by sampling 3.4-200 m^3 of air with Bendix Hurricane dual speed pumps (National Environmental Instruments, Inc., Warwick, RI 02888, Cat. No. 16003) at flow rates of 0.1-0.5 m^3/minute.

2. Descriptions of the Sampling Modules

a. The SURC sampling module is shown assembled (a) and exploded (b) in Figure 2. The basic module consists of a 4-inch (i.d.) by 2-inch (i.d.) (10 cm x 5 cm) glass process pipe reducer (Kimax 6650, size 4, or equivalent - Part 1 in Figure 2). This part is approximately 18 cm long. Standard glass pipe fittings (parts 2 and 3) are used on each end (2-inch and 4-inch connectors). At the smaller end, a stainless steel screen (3.9 openings/cm^2) is cut to fit and installed to hold the polyurethane foam plug (4) or other sorbent in place. A piece of stainless steel screen (1.5 openings/cm^2) is cut and installed at the larger end. This holds either the glass fiber filter (5) in normal operation or wool felt filter for controlled introduction of vapors of the test compounds. When foam is used as the sorbent, the larger opening screen may be used on both ends. The SURC module is shown in place on the sampler in Figure 1. The lower pipe fitting (part 3) is tightened down on a 2 inch stainless steel flange.

b. EPA - SWRI Sampling System - The sampling system was developed by Southwest Research Institute and was substantially modified and improved by EPA to allow use of a variety of sorbent types. It is shown in Figure 3. The same sampler described under Item 1 above is used. There are two parts to the sampling system: the sampling module or cartridge and the air-tight cartridge holder.

Sampling cartridge - A 65 mm borosilicate glass tube is cut to 125 mm in length. An indentation is formed 20 mm from one end (bottom) to provide a rim to retain a 25 mesh or larger stainless steel screen to hold the sorbent. The cartridge can then hold a polyurethane foam plug, porous (macroreticular) beads or other solid sorbents, or liquid coated beads.

This entire cartridge can be placed in a Soxhlet extractor for removal of substances collected in air. Vacuum drying at 30°C to 40°C restores the sorbent for reuse within several hours. The cartridge is shown in Figure 3 (Part a).

Cartridge holder - The basic cartridge holder is shown both assembled (b) and disassembled in Figure 3. Part 2 screws down on to Part 1 and silicone rubber (GLC septum sheet stock, Supelco Catalog No. 2-04626) gaskets (c) on both ends form an air-tight seal. Part 3, a 10-mesh stainless steel screen, holds either the glass fiber filter (d) or the felt pad. Part 4 holds the filter or pad in place. Part 1 is tapped and threaded on the bottom to attach to the 1/2 inch NPT inlet of the high volume air pumping system shown in Figure 1.

c. Bendix Hurrican Pump filter holder - The standard 10 cm filter holder is modified by attaching a cylindrical chamber, 25 cm long x 5 cm i.d., behind the filter holder with epoxy cement. Place two foam flugs, 5.5 cm diameter x 8 cm thick, in the chamber and a 10 cm diameter glass fiber filter in front of them in the filter holder. Connect the sampler to the Bendix pump by a 7.6 meter length of Flexaust CWC hose.

3. Calibration of Air Sampler

Refer to Figure 1 for a schematic diagram of the sampler. The needle valve is for test purposes only and is not used in normal operation. The red tap of the venturi goes to the high pressure side, and the green tap goes to the low pressure side of the Magnehelic gauge. A gasket goes between each floor flange and the base, and between the Hi-Vol housing and the base. Check these for leaks before starting.

Calibration procedure:

a. Attach the calibration venturi (Figure 4) in place of the sampling module and tighten securely.

b. Connect a 4"-0-4" slack tube Hg manometer to the taps of the calibrated venturi. Make sure the manometer is zeroed and level. Mark this manometer to indicate that it is to be used only with the audit venturi.

c. Zero the Dickson recorder (tap the face) and the Magnehelic gauge.

d. Turn the power transformer to 100 volts and turn the switch to ON. Allow the Hi-Vol motor to warm up for several minutes before readings are taken.

e. Record the ambient temperature in °C on the data form.

f. Record the barometric pressure in mm Hg.

g. Open the ball valve fully (the pointer on either zero mark). Record the audit venturi DP, the Dickson reading, and the Magnehelic reading.

h. Close the indicator valve slightly until the Magnehelic drops approximately 5 inches (13 cm) and record a new set of readings.

i. Repeat step (h) until five spaced sets of readings are obtained.

j. Remove the calibrated venturi.

k. Prepare a calibration chart of flow rate versus meter readings as shown in Figure 5.

To calibrate Bendix high volume pumps, force the exhaust air through a restricting orifice (supplied with the pump) and measure the resulting back pressure by a gauge placed directly ahead of the orifice. A gauge calibrated by the manufacturer to read directly in ft^3/minute air flow is available.

4. Descriptions of Sampling Media (Sorbents)

Two types of sampling media are recommended for use with the modified SURC sampler: polyurethane foams and granular solid sorbents. Foams may be used separately or in combination with granular solids in either sampling module described previously. With the EPA-SURC module, the sorbent may be extracted and reused (after drying) without unloading the cartridge.

Polyurethane foam (PUF) - Use polyether-type polyurethane foam [density No. 3014 (0.0225 grams/cm^3), or equivalent]. This is the type of foam generally used for furniture upholstery, pillows, and mattresses. It is white and yellows on exposure to light. Use 7.6 cm (3 in.) sheet stock and cut from it cylindrical plugs that fit under slight compression in the glass cartridge or module, supported by the wire screen. For the SURC module, the plugs should be 5.5 cm in diameter and fitted into the lower 5 cm (i.d.) chamber of the module. For the EPA-SWRI cartridge, the plug diameter should be 6.0 cm.

Granular solids - Porous (macroreticular) chromatography sorbents are recommended. Examples are Chromosorb 102, 20 to 40 mesh (Johns-Manville, Denver, CO); Porppak R, 50 to 80 mesh (Waters Associates, Milford, MA); Amberlite XAD-2, 16 to 50 mesh (Rohm and Haas Co., Philadelphia, PA); Tenax - GC, 60 to 80 mesh (Enka N. V., The Netherlands); and Florisil PR-grade, 16 to 30 mesh (Floridin, Pittsburgh, PA). Pore sizes and mesh sizes must be selected to permit air flow rates of at least 200 liters/minute. Approximately 25 cm^3 of the sorbent is recommended. The granular solids may be "sandwiched" between two layers of polyurethane foam (a 60 mm diameter x 50 mm foam plug on top and a 60 mm diameter x 25 mm PUF plug on the bottom) to prevent loss during sampling and extraction (Figure 6).

5. Preparation of Sampling Media

   a. Prepare sorbent for initial cleanup before use. For foam, cut an appropriate size cylindrical plug with a cutting tool and place in a Soxhlet extractor. For granular or porous polymeric solids, add to pre-extracted Soxhlet thimble and place in the extractor.

b. Extract with 5% diethyl ether in n-hexane (glass distilled, pesticide quality or equivalent) or other appropriate solvent(s) for 14-24 hours at ca 4 cycles/hour.

NOTE: To determine the blank value of each plug, extract twice for periods of 7-12 hours; concentrate the second solvent, pass through an alumina column, and analyze by GLC (see Section 8,B).

c. Dry the sorbent under vacuum at 75°C.

d. Place the sorbent into glass sampling modules. For loose solids, the appropriate volume (e.g., 25 ml) should be measured and the corresponding weight recorded.

e. Place the sampling module in a sealed container or wrap in hexane-rinsed aluminum foil until ready for use.

6. Determination of Sampling Efficiencies for Specific Pesticides

a. Pesticide Retention Efficiency

No air sampler may be used for assessing of atmospheric concentrations of any compound without first determining the efficiency of the sampler to trap and retain the compound. Determine retention efficiencies by multiple injections of microliter volumes of the pesticide of interest in n-hexane directly into the sorbent trap. After a one hour drying period, place the fortified trap in front of a second trap in the sampling system. Pump ambient air through the train for the length of time and volume to be used in the sampling (i.e., for the high volume system, 24 hours at 200-250 liters/minute) to determine breakthrough to the second trap. Exclude airborne particulate matter by means of a glass fiber prefilter.

b. Pesticide Collection Efficiency

Determine collection efficiencies by vaporizing individual compounds or mixtures into the intake of the air sampler under study. Replace the glass fiber prefilter with a pre-extracted wool felt filter (weight 14.9 mg/cm^2, thickness 0.6 mm), which is then fortified with the pesticide of interest before pulling ambient air through it and, subsequently, the vapor trap(s). Add dropwise hexane solutions containing microgram amounts of the test compounds to the filter in amounts of 1 ml or less, and evaporate the solvent before the filter is attached to the sampling module. After 24 hours of air flow, analyze the filter and sorbent trap(s) individually (Section 8,B). Make at least one blank determination with unfortified filters simultaneously to correct for airborne interferences and possible contamination or losses from the analytical methodology.

Perform these tests outdoors with unaltered ambient air (in a rural, nonindustrialized area) whenever possible. When required, filter the intake air through a PUF trap to remove interfering contaminants.

All pesticidal compounds used for establishing sampling efficiency should be of the highest purities obtainable. Purities should be checked before use. All solvents should be of pesticide quality or equivalent.

Conduct at least six independent trials for each test compound in order to provide statistical data. Acceptable standard deviation values will depend on the nature of the pesticide. For example, for the less volatile, more chemically stable, and more easily analyzed pesticides, higher precision and accuracy of results will be expected.

A sampling efficiency of 75% should, in general, be considered satisfactory for a collection medium. For the more easily trapped pesticides such as DDT and mirex, sampling efficiencies should be essentially quantitative. Reuseability of sorbents is considered important; as a guideline, at least six months of repeated use should be expected before loss in sampling efficiency is noted. The sorbents selected are also expected to vary little in trapping and retaining test compounds with changes in temperature and humidity.

7. Collection of Air Samples

A modified SURC air sampler may be operated at ground level or on roof tops. In urban or congested areas, it is recommended that the sampler be placed on the roof of a single-story building. The sampler should be located in an unobstructed area, at least two meters from an obstacle to air flow. The exhaust hose should be stretched out in the downwind direction, if possible. The sampler should be operated for 24 hours in order to obtain average daily levels of airborne pesticides. (Air concentration may fluctuate with time of day, temperature, humidity, wind direction and velocity, and other climatological conditions.) On and off times and weather conditions during the sampling period should be recorded. Air flow readings should be taken (from the Magnehelic gauge) at the beginning and end of each sampling period. The chart from the Dickson recorder should be examined to note the occurrence and duration of any power failure and any change in sampling rate during the period. Blower motor brushes should be inspected frequently and replaced, as necessary. An electrical power source of 110 VAC, 15A is required.

The glass sampling cartridge and glass fiber filter (on the modified SURC module) should be removed from the sampler with forceps and clean, gloved hands and immediately placed in a sealed container(s) for transport to the laboratory. Similar care should be taken to prevent contamination of the filter and vapor trap when loading the sampler.

8. Results and Discussion

The greatest value of the high volume collection system is that it provides a large sampling of air (at least 300 m^3/24 hours). Thus, even with poorly trapped compounds, sufficient quantities can be collected to detect very low air concentration. For efficiently collected compounds, detection limits can be extended to the subpicogram-per-cubic-meter range, and sufficient quantities can often be trapped in 24 hours to provide for mass spectrometric confirmation.

See Section 8,B for collection data on pesticides and PCBs.

III. SOURCE SAMPLING:

Contaminated, or source-related, atmospheres generally present less problems with respect to either the sampling process or analytical measurement because of the higher levels of pesticide present. However, source sampling often requires special sampling equipment that is portable, battery-powered, or is otherwise commensurate with specific sampling needs. Often it is also not practical (or desirable) to collect 24-hour samples. Thus a relatively high-flow device, which may also need to be portable and/or battery operated, may be required.

Monitoring atmospheres inside domiciles or workplaces requires a sampler that is unobtrusive and operates quietly, does not get in residents' or workers' way, and places little or no time or financial demands on the site owner to maintain. Similar requirements are made on devices used to monitor inspired air. They need to be worn on the person; hence, must be battery-operated, light weight, comfortable and quiet. Ideally, they should sample air at flow rates similar to normal human respiration. Since indoor levels are generally much higher than outdoor levels, due mainly to pest control measures exercised inside domiciles and places of employment, small, low volume air samplers may be used. Sampling rates in the 1 to 10 liters/minute range are adequate and can be provided with any of several personal air sampling pumps on the market. These pumps can be operated on batteries for up to 8 hours or for longer periods if attached via a charging unit to 110 VAC house current. Personal sampling devices are discussed further in Section IV.

1. High Volume Source Sampler

   A high volume sampler developed for the U. S. Army and manufactured by Environmental Research Corporation (ERCO), a subsidiary of Dart Industries, St. Paul, MN, is shown in Figure 7. Air is drawn at flow rates up to 185 liters/minute through either or both of two parallel 15 cm composite filter pads comprised of Poropak R sandwiched between two layers of glass fiber mat.

   The major advantage of the ERCO sampler for source air monitoring is that is provides a relatively large sample size with short sampling times. It is compact and light weight, which makes it highly portable. The model studied was equipped for either AC or DC power and could be operated on a heavy duty automobile battery at flow rates up to 160 liters/minute. The greatest disadvantage of the system is the high cost of the composite filter pads, which cannot be reused.

2. Low Volume Indoor Source Samplers

   a. Pumps:

      MSA Monitaire™ Sampler, Model S, Catalog No. 458475 and charger No. 456059. Mine Safety Appliances Company, 600 Penn Center Boulevard, Pittsburgh, PA 15235

      or

      DuPont Constant Flow Sampling Pump, Model P4000A (includes charger), Catalog No. 66-241 (Figure 8). DuPont, Applied Technology Division, Wilmington, DE 19898.

      Both of these small, battery operated pumps are capable of pumping air through an 18 mm diameter x 50 mm cylindrical PUF plug at 2.5 to 4 liters/minute for at least 8 hours with a fully charged battery pack. The DuPont pump has the advantage that it will automatically adjust its pumping rate to compensate for changes in flow resistance (e.g., due to accumulation of particulate matter at the intake of the collection module). It also operates more quietly than the MSA and can be programmed to stop sampling after a prescribed period.

b. Collection Devices:

Any cartridge capable of holding a cylindrical plug of polyurethane foam (approximate volume 15 to 20 cm^3) or 5 to 10 cm^3 of granular sorbent can be used. Several collection modules are shown in Figure 9, along with a portable pump.

Module a is a Teflon bottle containing a 2.5 cm diameter x 5 cm foam plug preceded by a 2 cm diameter glass fiber filter (Gelman Type A or MSA CT-75428) mounted in the cap. The hose attachment is constructed from a plastic hose connector pressed tightly through a hole in the bottom of the bottle and sealed with Teflon tape.

Module _b_ is an open glass tube, 18 mm i.d. x 50 mm, drawn down to a 7 mm o.d. open tip on one end for attachment to the plastic tubing. The foam plug is cut slightly oversized for a compression fit. This module has no provision for separate collection of particulate matter.

Module _c_ is a standard filter holder (e.g., MSA No. 92944) for dust collection only. Either glass fiber (Gelman Type A or MSA CT-75428) or PVC membrane filters (e.g., MSA Type FWS-B), 37 mm in diameter, may be used.

Modules _a_ and _b_ are most suitable for use with granular sorbents. It is suggested that small cylinders of polyurethane foam be inserted before and after the granular sorbent to retain the latter in place.

A glass tube, 2 cm in diameter and 7.5 cm long (tapered the last 3 cm) has also been used for the sampling cartridge with MSA and DuPont pumps. A small foam plug, 4 cm long x 2 cm diameter is placed in this tube, and the entire tube is wrapped in hexane-rinsed foil for transport. For sampling, the tube is connected to the MSA pump by a length of Tygon tubing.

c. Preparation and Analysis of Sorbents and Glass Fiber Filters

Follow the same basic procedures described in Subsection II,5. Scale down volumes for the smaller plugs or quantities of granular sorbents used. Smaller Soxhlet extractors will cycle more frequently (e.g., 8 cycles/hour). Because efficient extraction of pesticide from glass module b (Figure 9) will probably not be achieved with the sorbent in place, extract the foam and sorbent separately. Cut glass fiber filters to size, wrap loosely in aluminum foil, heat to 315°C in a muffle furnace overnight to remove any organic material, and place in a desiccator until use.

d. Calibration of Air Sampler

For low volume samplers, a simple soap bubble meter is adequate for calibration. The commercial calibration unit shown in Figure 10 is available from Mine Safety Appliances (Catalog No. 457629). It consists of a one liter bubble tube assembly, manometer, needle valve, stop watch, and voltmeter (for battery test).

When polyurethane foam alone is used, as in module b, (Figure 9), the sampling pump may be calibrated without the module attached. However, the additional use of a prefilter or granular sorbents causes sufficient pressure drops across the sampling module to require calibration with the loaded module in place. In all cases, it is suggested that the loaded sampling module be installed during calibration, or there may be very large differences between the pump flow meter reading and actual flow achieved through the module.

For calibration, the pump and sampling module should be attached to the top of the bubble meter upstream of the needle valve and manometer (location f in Figure 10). Some means of adapting the intake face of the module into the calibration system must be devised. For module b, laboratory "bubble" tubing (3/4 to 3/8 inch) may be used. (A suggested source of the latter is Sherwood Medical Industries, Argyle, NY.) Allow the pump to operate a few minutes, then set the manometer to read 2 inches (5 cm) of vacuum by adjusting the needle valve. Squeeze the rubber bulb at the bottom of the bubble meter to introduce soap bubbles. Use the stop watch to time the passage of soap bubbles between the calibration marks (one liter). Adjust the pump to achieve the desired flow rate or generate a plot of flow rate versus the pump rotameter reading.

The DuPont Company markets a calibration unit (Catalog No. 66-242-f-1) especially designed for the Model P4000A pump. It includes a bubble tube, flow rate meter, and pressure drop meter (Figure 11).

Pumps may also be calibrated by displacing water from an inverted 2 liter graduated cylinder during a measured length of time.

e. Determination of Sampling Efficiencies for Specific Pesticides

Measured quantities of pesticides in a volatile solvent such as n-hexane are placed in a U-tube, which is attached to the sampling module. The U-tube is immersed in a heating bath, which is carefully controlled to slowly volatilize the pesticide. After the sampling period (which whould be as long as that anticipated in actual monitoring studies), the amount of pesticide remaining in the U-tube and that collected by the sorbent is determined to establish collection efficiency. Sampling periods should be 4 to 8 hours.

f. Collection of Air Samples

For determination of pesticide residues indoors, air samples should be taken in as many locations as necessary to achieve a profile of the distribution throughout the building. In houses with forced-air heating and/or air conditioning, air concentrations will tend to be equilibrated, although there will probably be areas in rooms where circulation is impaired. Unlike the situation in outdoor air, there should be little diurnal variation in pesticide levels. Concentrations may vary more widely in houses without air circulating devices, and may also be weather dependent (i.e., depend on whether windows and doors to the outdide are open or closed).

Nearly all domiciles and many other buildings are given preconstruction termite treatment. This results in a slow release of the insecticide over very long periods of time (at least up to 25 years). In buildings where circulation is poor, airborne termitacide levels may be higher in basements or ground floors than on other floors. In "plenum houses", the crawl space under the house serves as the plenum in the air destribution system, which contributes substantially to the transport of termitacide to other portions of the dwelling.

Kitchens and bathrooms are favorite areas for insects such as roaches, ants, and silverfish; consequently, the application of insecticides in these areas is common practice. The chemicals are usually applied in baits or in slow-release formulations, so that pesticides may be emitted into the air for many months after treatment. Similarly, crack and crevice treatment for pest control is popular in commercial buildings.

The design of the structure and history of its past control treatment should be taken into account when planning an air monitoring project. Several samplers should be used at once to obtain a distribution profile of pesticide levels in the building. Normally, an 8 hour sampling period at about 2 liters/minute is sufficient to obtain an adequate sample for analysis. In this period, about one m^3 of air is sampled, which should provide a detection limit of 0.1 $\mu g/m^3$ or lower for most pesticides. This level is one-tenth that of the National Institute of Occupational Safety and Health proposed standard of 1 $\mu g/m^3$ for a 10 hour work day, 40 hour work week exposure to carcinogenic compounds. Although the portable pumps described earlier in this section are designed to operate for 8 hours on fully charged battery packs, house current (through the battery charger) should be used when available to assure more uniform pumping rates during the sampling period.

The air intakes of the sampling modules should be placed one or two meters above floor level and oriented downward or horizontally. If oriented upward, non-respirable pesticide loaded dust may be collected. If pesticide residues on household dust particles appear to be very significant, a prefilter should always be used.

IV. WORKPLACE AIR - PERSONNEL MONITORING:

Inhalation of airborne dust and vapors containing high concentrations of pesticides constitutes a serious hazard to pest control operators, pesticide formulators, and other persons occupationally involved in agricultural industry. Respiratory exposure can be best assessed through the use of a personal monitor worn on the body while working in areas of high pesticidal contamination.

1. Air Sampling Devices

The small sampling units described in the previous section are designed for personal monitoring. They are battery operated and can be worn on the body.

The MSA pump weighs 870 grams and may be worn comfortably on the waist belt. The DuPont pump is also designed to be attached to a waist belt, but is rather heavy (1.2 kg with battery packs that are required for 8 hours of operation). DuPont markets a smaller constant flow unit that weighs only 400 grams, but it draws only 200 ml/minute at full flow (no resistance). In order to achieve the sensitivity in the 0.1 to 1 $\mu g/m^3$ range for many pesticides, flow rates of 1 to 3 liters/minute are needed, particularly for sampling periods that are necessarily shorter than 8 hours. The sampling modules are attached to the shirt collar or lapel to monitor air in the breathing zone. The intake should be oriented downward to exclude large dust particles, which may not enter the nostrils.

It has been pointed out that estimation of respiratory exposure in areas of high pesticide concentration is accurate only for true gases, due to the probable lack of uniform dispersion of particulate matter in the breathing zone. The aerodynamics of respiration through the nostrils is difficult to duplicate with an air sampler. Most sampling devices also will not differentiate between particles that would be trapped in the nasopharynx and the smaller respirable particles that reach the lungs. However, a small cyclone sampler that separates and discards nonrespirable particulates (above 10 μm in diameter) is marketed by Mine Safety Appliances. The unit, called the Gravimetric Dust Sampling Kit (MSA 456241), can be attached to the collar or lapel and is designed to sample at three calibrated flow rates (2.0, 1.8, and 1.6 liters/minute). Respirable particulate matter collected in a filter cassette may be analyzed for pesticide content. A separate vapor trap (and pump) could be worn for comparative data.

2. Preparation and Handling of Samples

Pre- and post-treatment of sampling devices and analytical procedures should be identical to those described in the preceding sections. Special care should be exercised to avoid contamination of samples in the field. Improper handling of the collection module before or after the sampling period could easily deposit a microgram of the material being monitored (or interfering substance) on the sampling medium, which would result in a false positive analysis of 1 $\mu g/m^3$. Therefore, the collection modules should be loaded in the laboratory and sealed in hexane-rinsed aluminum foil or a clean, sealed glass jar before transport to the field. An analyst should carefully install the sampling device and instruct the wearer not to touch or disturb it. The analyst should be present at the end of the sampling period to remove the module, place it in a sealed container, and transport it back to the laboratory.

3. Sampling times should be commensurate with known or anticipated exposure times. If potential exposure to airborne pesticides is intermittent or brief, sampling should be performed only during those periods. If exposure is continuously uniform throughout the work day, sampling may be conducted for only a portion of the day and the result extrapolated to estimate the total exposure for the entire work period. If exposure is not uniform but occurs for regular periodic cycles during the work day, sampling should be conducted over the entire work day to obtain an accumulated total exposure assessment. The monitoring program selected should, of course, be the result of careful planning in order to provide a realistic assessment of worker exposure.

4. Estimation of Inspired Quantities

Since the sampling rates achievable with small, battery operated pumps are substantially lower than the respiratory rates of most workers, monitoring data must be extrapolated on the basis of estimated lung ventilation values to obtain an assessment of total exposure. Table 1 gives the average respiratory rates and their normal ranges for men and women at rest and at work. Values would be lower for children and elderly people. Ideally, pulmonary function test (PFT) measurements should be made on the worker while performing the job in order to determine the exact respiratory rate. Since PFT equipment and personnel trained in its operation are not likely to be available, a subjective estimation must be made of the breathing rate if an approximation of the total quantity of pesticide inspired is desired. To the untrained eye, it may sometimes be difficult to differentiate between light and heavy work. Estimates of average respiration rates likely to be encountered among persons occupationally exposed to pesticides have been made by H. R. Wolfe based on many visual observations over many years. These estimates, which are given in Table 2, are subjective but may be better than inexperienced judgments. They should not be used *a priori* unless the data are appropriately qualified. Also, unless the sampler used can differentiate between respirable and nonrespirable particulate matter, it cannot be assumed that the quantity of pesticides collected is proportional to the total inspired into the lungs.

TABLE 1. NORMAL RESPIRATORY RATES FOR HUMANS

Respiratory Rates in Liters per Minute

Level of Activity	Adult Male		Adult Female	
	Avg.	Range	Avg.	Range
Rest	7	6-10	4	4-7
Light Work	29	27-31	16	16-17
Heavy Work	60	50-90	24	17-32

TABLE 2. SUBJECTIVE ESTIMATIONS OF RESPIRATORY RATES FOR PESTICIDE WORKERS

Work Situation	Estimated Respiratory Rate (L/min)	
	Adult Male*	Adult Female**
Agricultural Workers:		
Sprayer Using Hand Gun and Dragging Hose	50-67	22-25
Driver of Tractor Pulling Spray Equipment	18	10
Fruit Thinner or Picker	29-30	16
Flagger for Aircraft Spray Application	18	10
Pesticide Formulation Plant Workers:		
Bagger (Filling small bags - 2 to 5 lb.)	29-30	16
Bagger (Filling large bags - 50 lb.)	32-33	17
Stacker (Stacks 50 lb. bags or pallets)	33-42	17-20
Bagger & Stacker (Filling and stacking 50 lb. bags or pallets)	33-42	17-20
Boxer (Packing small bags into shapping boxes)	30-32	16-17
Fork Lift Operator	20	12
Mixer (Emptying bags of dry pesticide into hopper for blending)	33	17
Worker Cleaning Inside of Hoppers and Bins	33	17

*Based on numerous visual observations and the respiratory rates given in Table 1.

**Calculated as the percent of the male rate using data in Table 1.

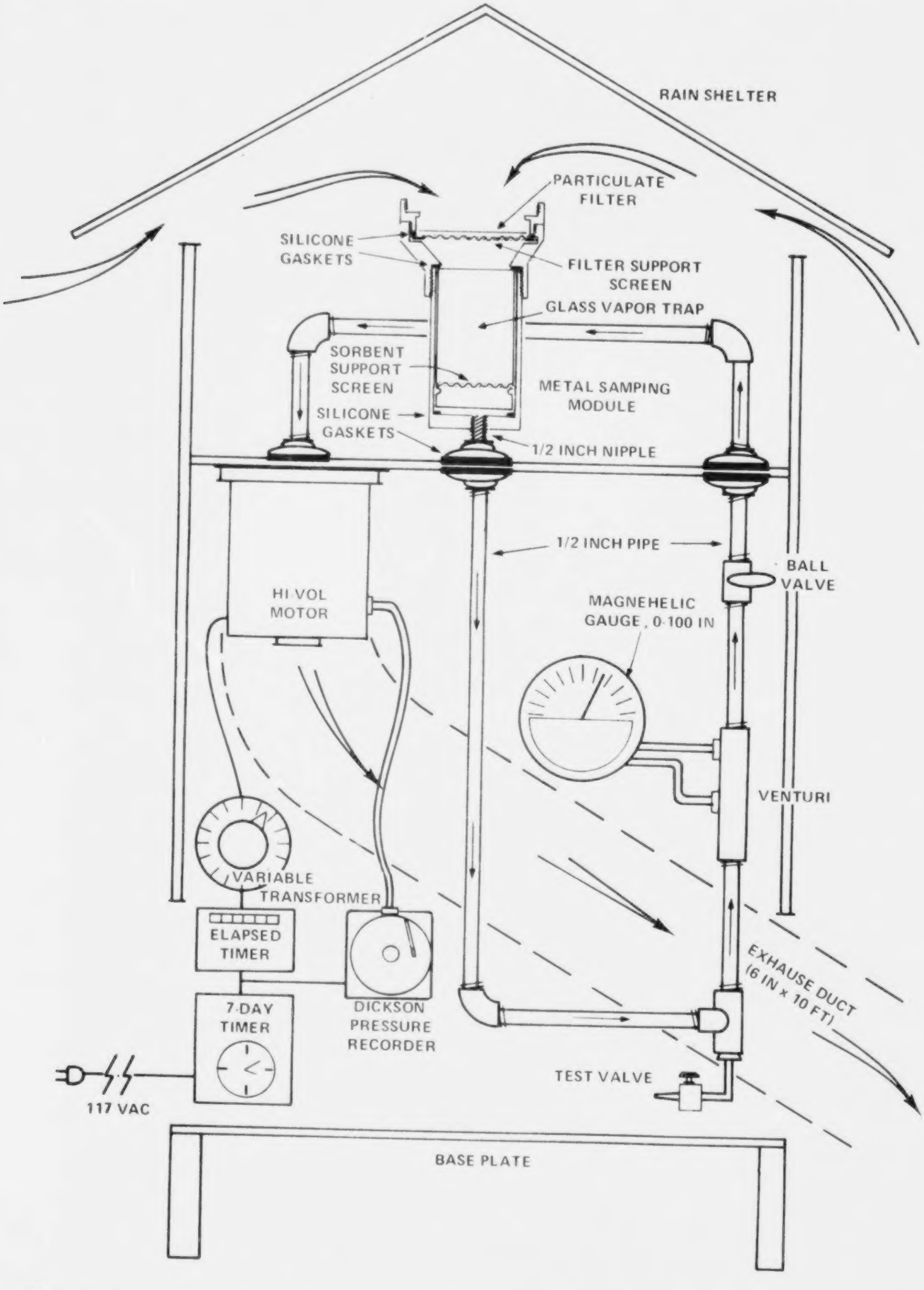
RAIN SHELTER
PARTICULATE FILTER
SILICONE GASKETS
FILTER SUPPORT SCREEN
GLASS VAPOR TRAP
SORBENT SUPPORT SCREEN
METAL SAMPING MODULE
SILICONE GASKETS
1/2 INCH NIPPLE
1/2 INCH PIPE
BALL VALVE
HI VOL MOTOR
MAGNEHELIC GAUGE, 0-100 IN
VENTURI
VARIABLE TRANSFORMER
ELAPSED TIMER
DICKSON PRESSURE RECORDER
7-DAY TIMER
EXHAUSE DUCT (6 IN x 10 FT)
TEST VALVE
117 VAC
BASE PLATE

Figure 1. EPA high volume ambient air sampler for pesticides, PCBs and other organic compounds.

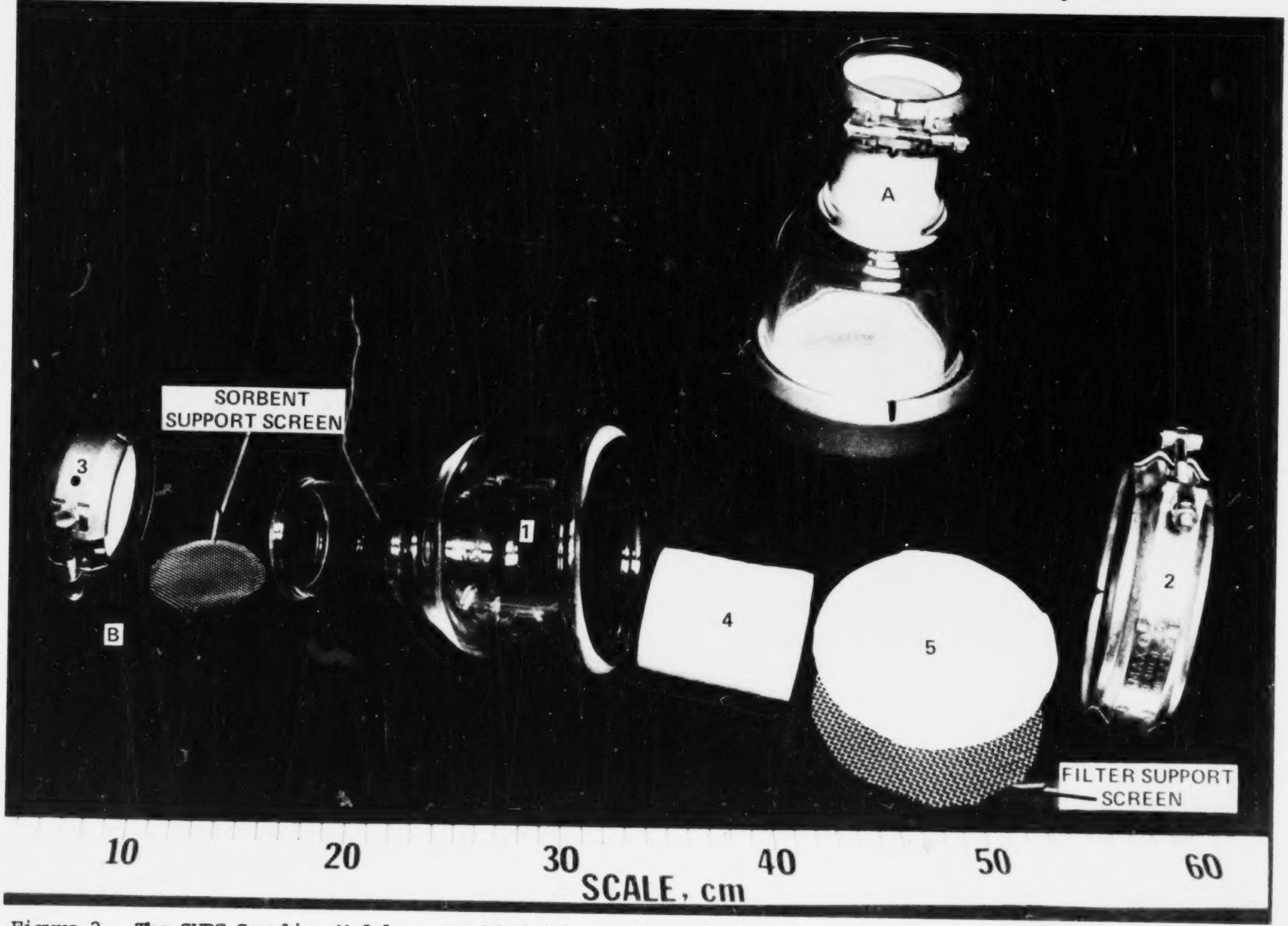
A
SORBENT SUPPORT SCREEN
3
B
1
4
5
2
FILTER SUPPORT SCREEN
10
20
30
40
50
60
SCALE, cm

Figure 2. The SURC Sampling Module, assembled (a) and disassembled (b). Part 1 is a 4-in x 2-in glass process pipe reducer. Parts 2 and 3 are stainless steel pipe fittings with Teflon inserts. Part 4 is a 5.5-cm x 7.6-cm polyurethane foam cylinder and Part 5 is a Gelman Type A glass fiber filter (it is installed under Part 2).

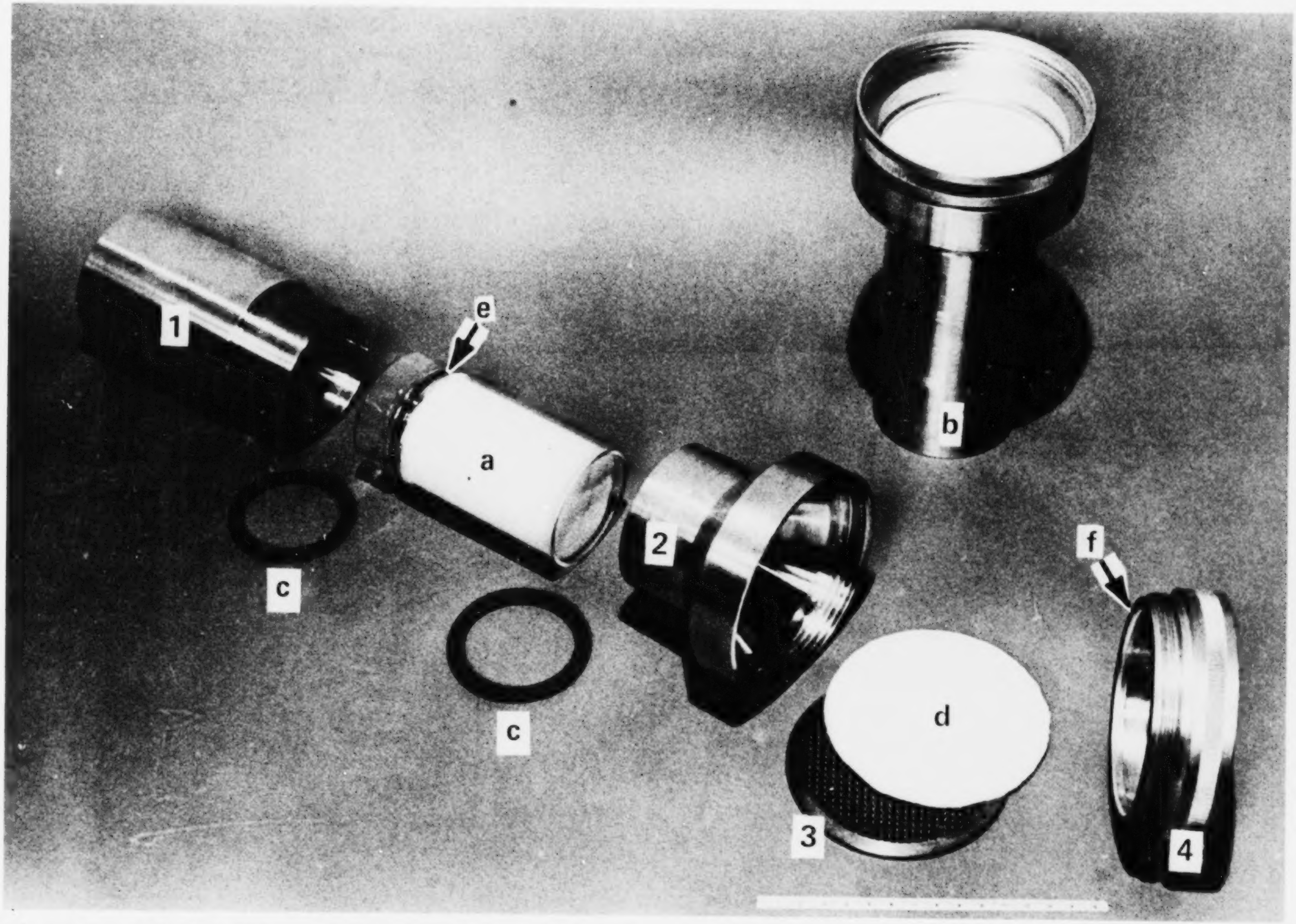

Figure 3. EPA High-Volume Sampling Module. (a) Sampling cartridge; (b) Assembled module containing cartridge and prefilter; (c) Silicone rubber gaskets; (d) Glass fiber pre-filter; (e) Support Screen; (f) Silicone rubber "O"-ring. Part 1 - Cartridge receptacle. Part 2 - Prefilter adapter. Part 3 - Filter support screen. Part 4 - Filter retaining ring.

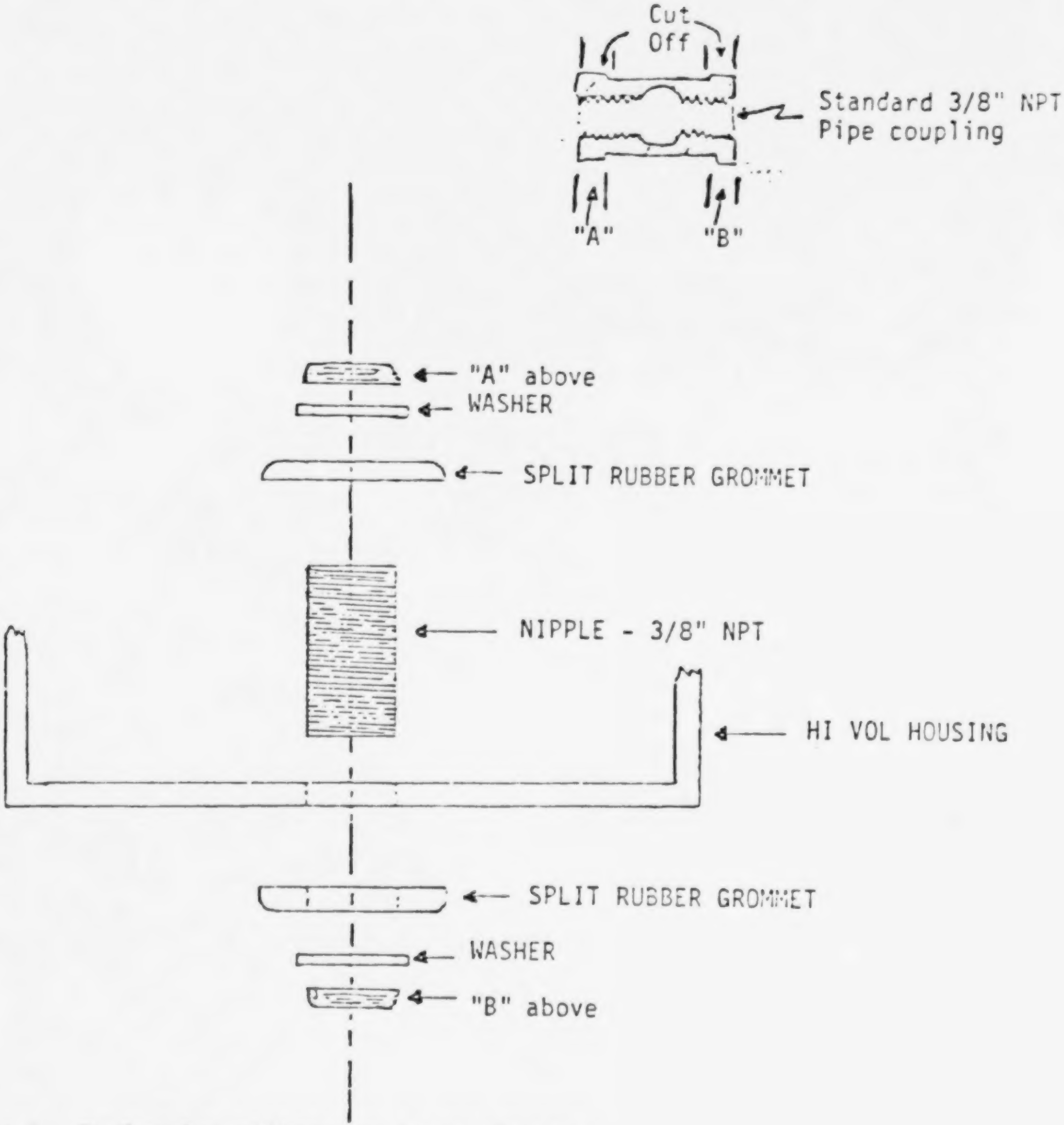
Cut Off
Standard 3/8" NPT Pipe coupling
"A"
"B"
"A" above
WASHER
SPLIT RUBBER GROMMET
NIPPLE - 3/8" NPT
HI VOL HOUSING
SPLIT RUBBER GROMMET
WASHER
"B" above

Figure 4. Device for calibration of Air Sampler.

NOTE: NOT DRAWN TO SCALE

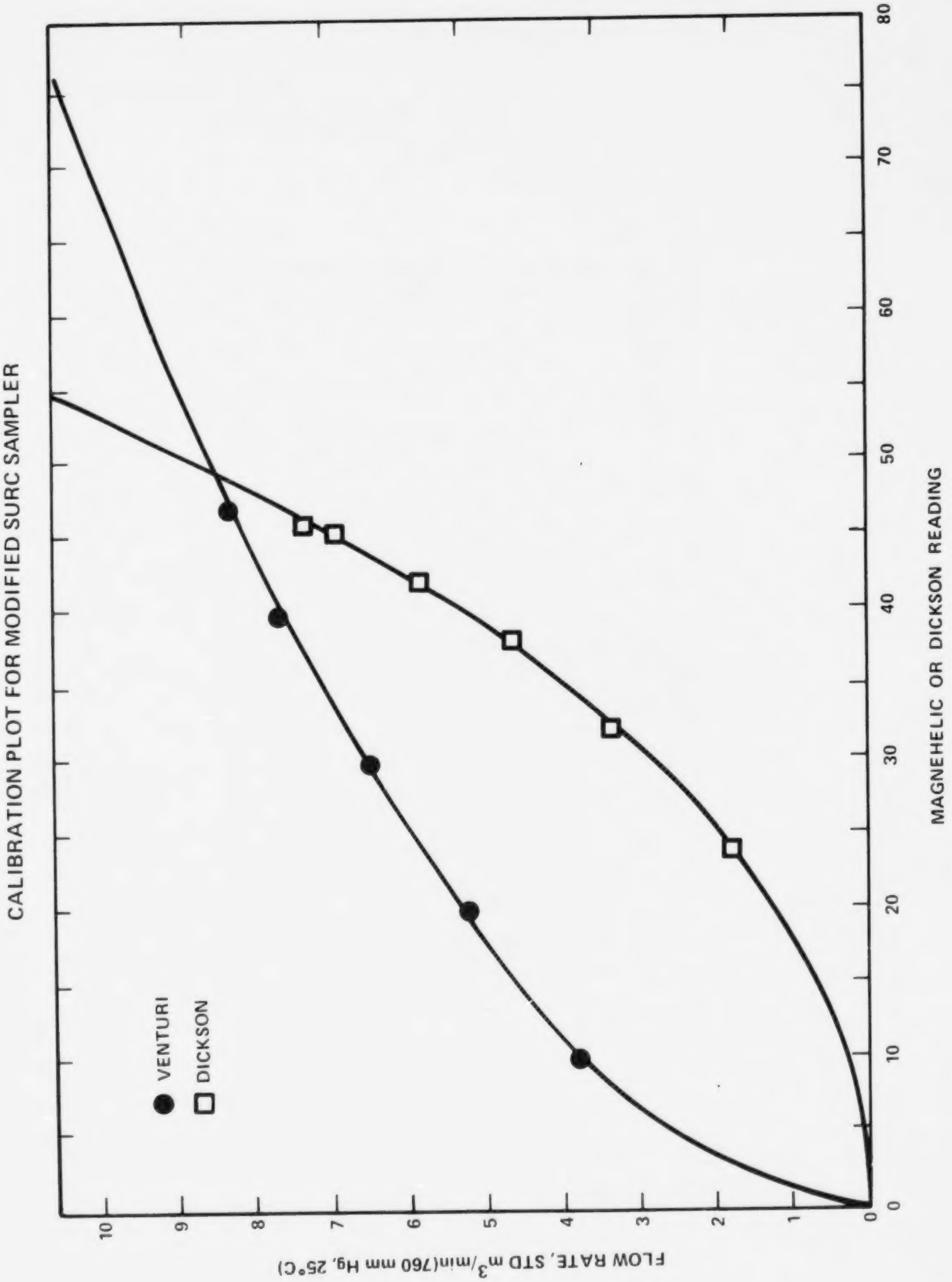

Fig 5. Calibration plot for modified SURC sampler.

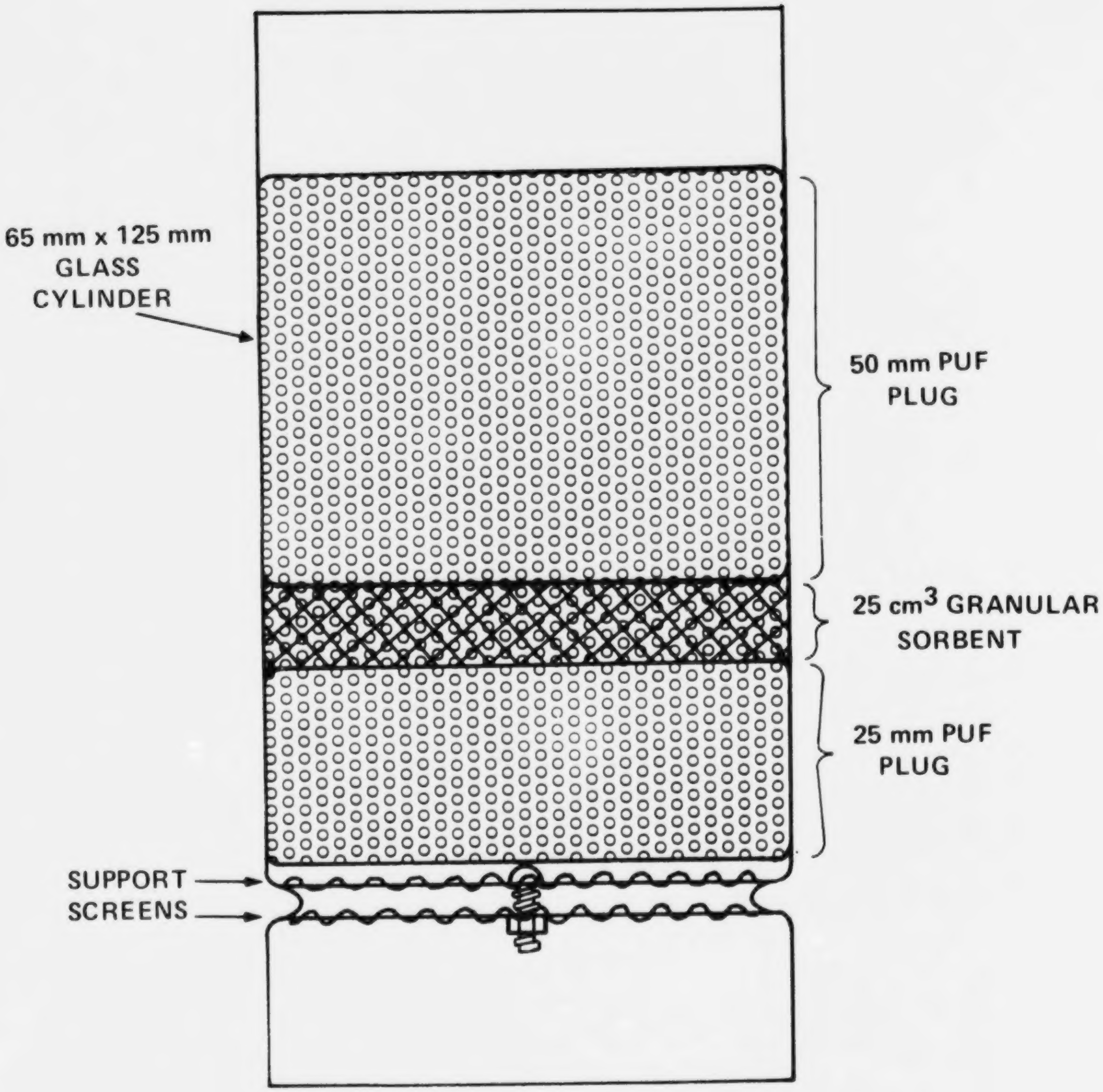
65 mm x 125 mm
GLASS
CYLINDER
50 mm PUF
PLUG
25 cm3 GRANULAR
SORBENT
25 mm PUF
PLUG
SUPPORT
SCREENS

Figure 6. Dual sorbent vapor trap.

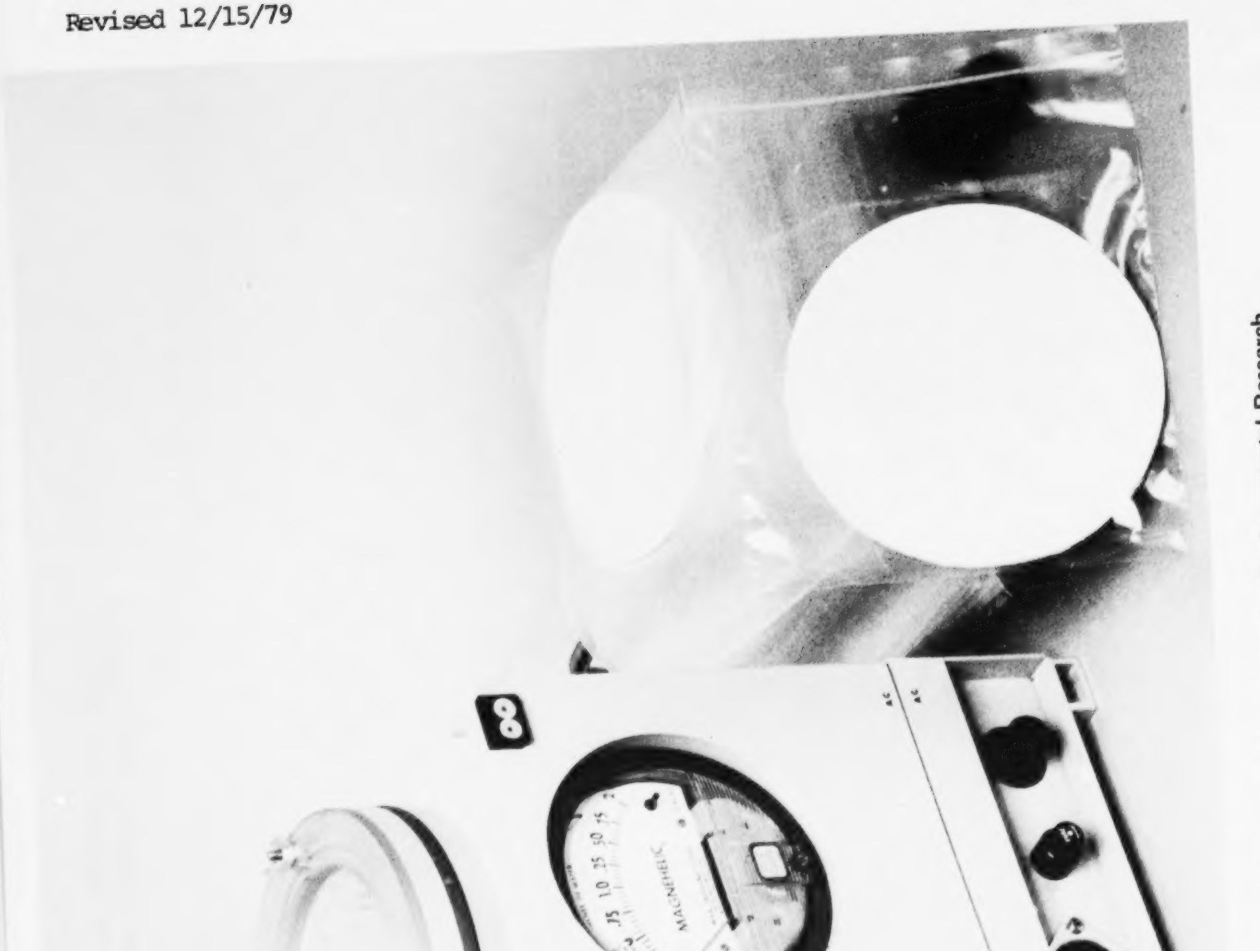

Figure 7. A high-volume air sampler developed for the U. S. Army by Environmental Research Corporation (ERCO). Shown to the right are the composite filter pads used to trap airborne pesticides.

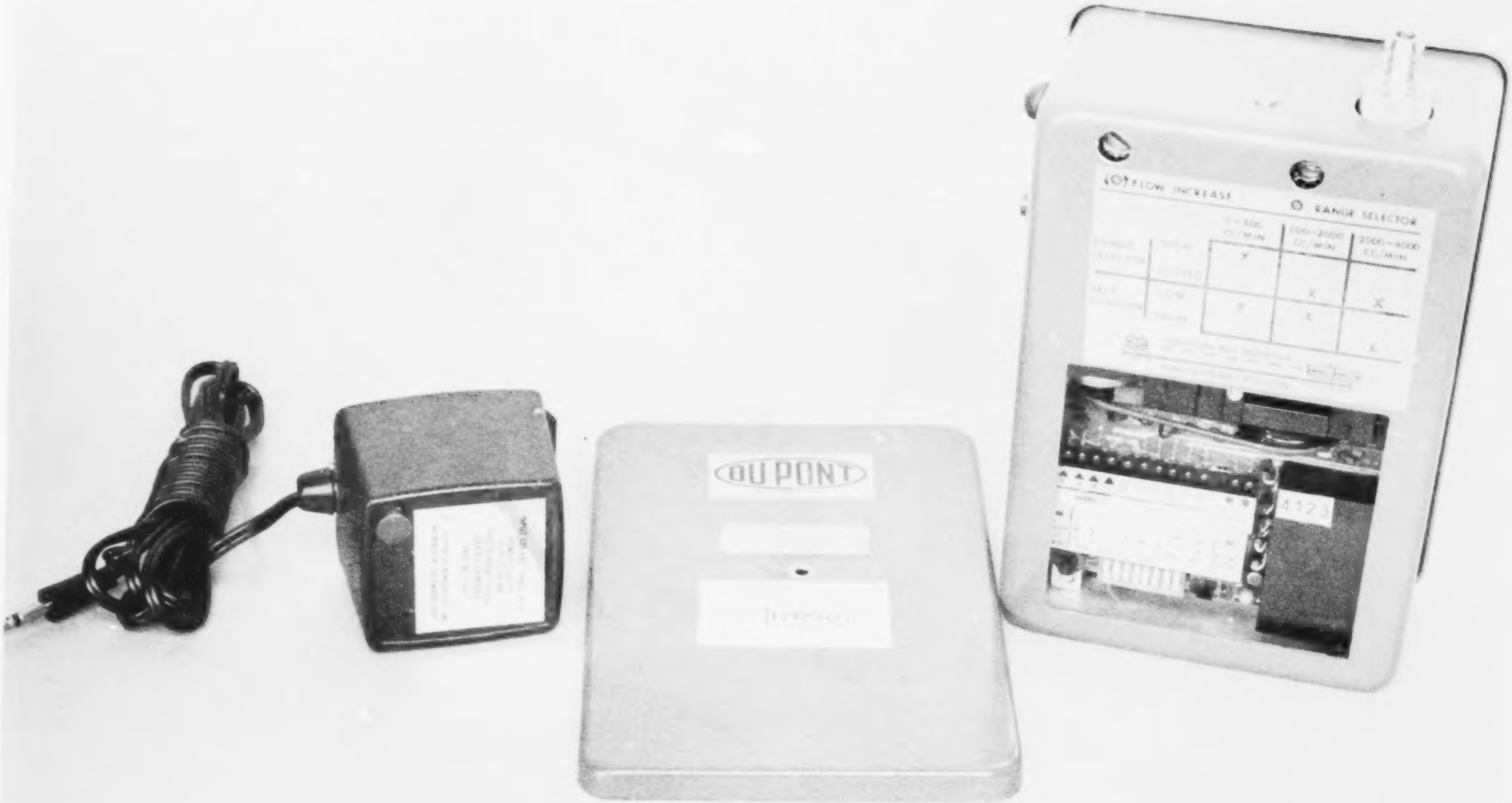
FLOW INCREASE
RANGE SELECTOR
0-300 CC/MIN
300-2000 CC/MIN
2000-4000 CC/MIN
DU PONT
4123

Fig. 8. The DuPont Constant Flow Sampling Pump (cover removed) and battery charger.

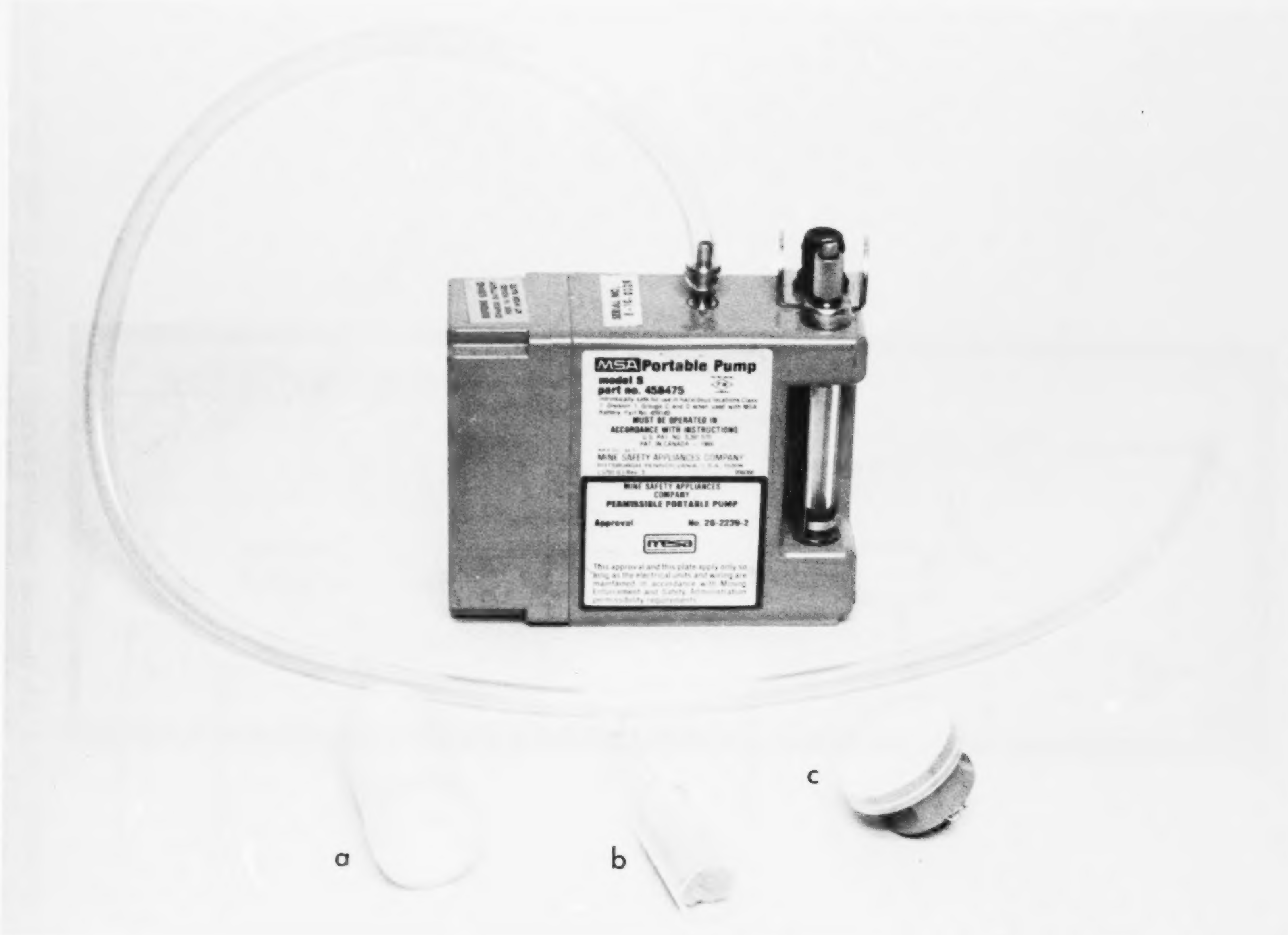
MSA Portable Pump
model S
part no. 458475
MUST BE OPERATED IN
ACCORDANCE WITH INSTRUCTIONS
MINE SAFETY APPLIANCES COMPANY
MINE SAFETY APPLIANCES
COMPANY
PERMISSIBLE PORTABLE PUMP
Approval
No. 20-2239-2
mesa
a
b
c

Figure 9. Personal sampling pump and three collection modules. (a) Polyurethane foam with particulate filter in modified Teflon bottle, (b) polyurethane foam in glass holder, and (c) filter holder for dust collection only.

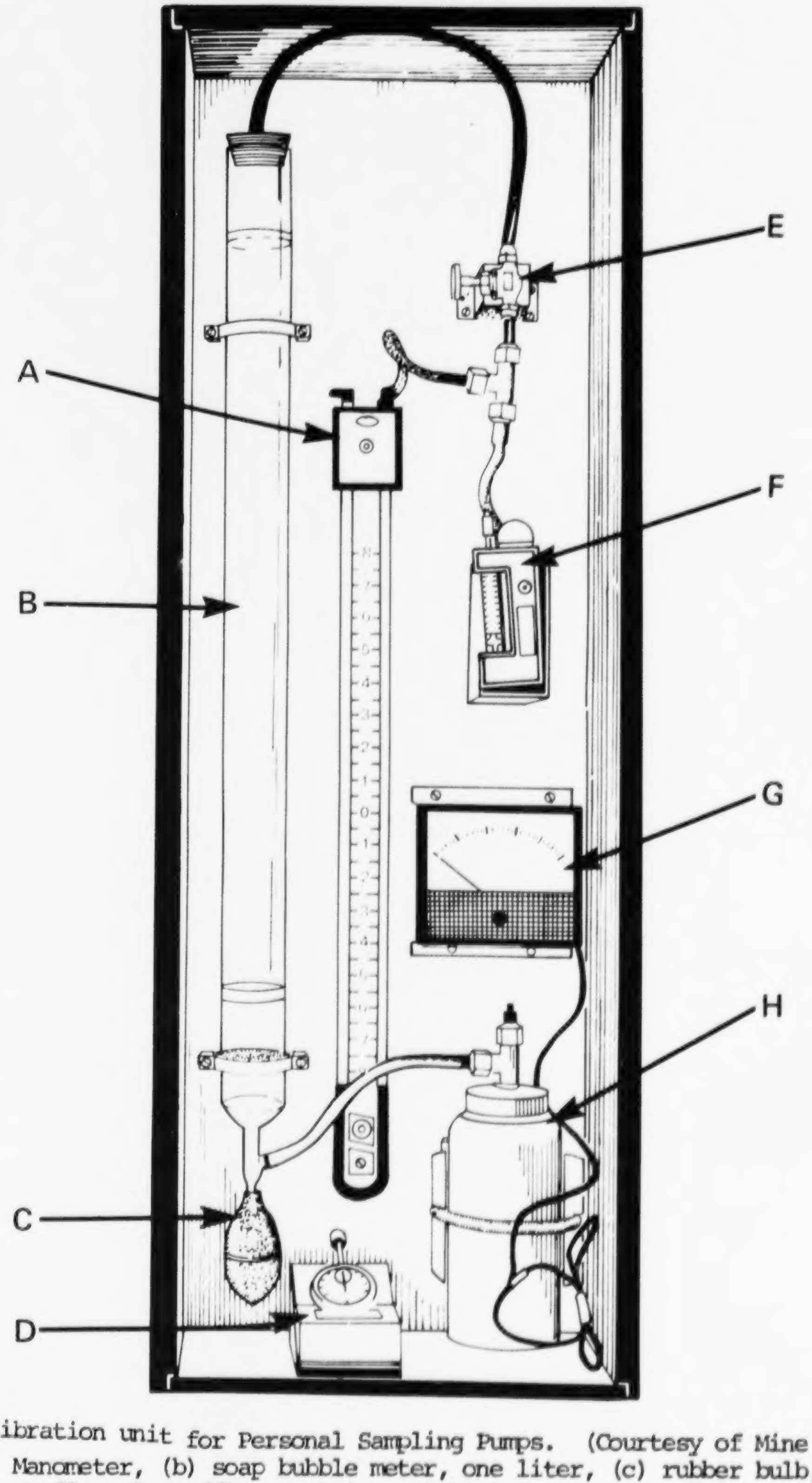

Figure 10. Calibration unit for Personal Sampling Pumps. (Courtesy of Mine Safety Appliances). (a) Manometer, (b) soap bubble meter, one liter, (c) rubber bulb, (d) stop watch, (e) needle valve, (f) pump being calibrated, (g) voltmeter, 0-10 V, and (h) soap solution reservoir.

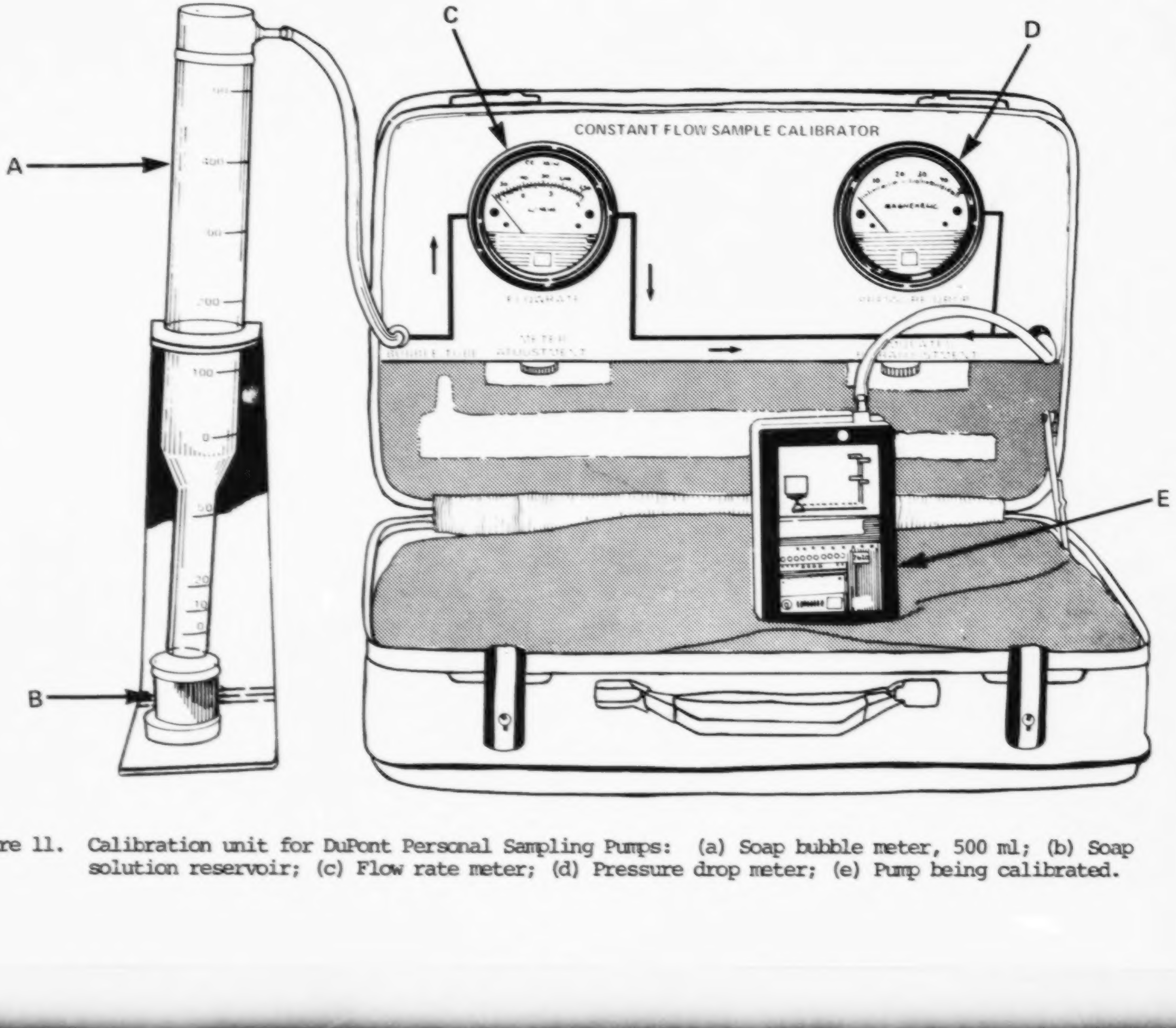
A
B
C
D
E
CONSTANT FLOW SAMPLE CALIBRATOR
400
200
100
0
50
20
10
0
BUBBLE TUBE
METER ADJUSTMENT
FLOWRATE

Figure 11. Calibration unit for DuPont Personal Sampling Pumps: (a) Soap bubble meter, 500 ml; (b) Soap solution reservoir; (c) Flow rate meter; (d) Pressure drop meter; (e) Pump being calibrated.

ANALYSIS OF PESTICIDES IN AIR

I. INTRODUCTION:

The analytical scheme described in this section presupposes collection of the samples by one of the procedures described in Section 8,A. The methodology replaces that reported in the last revision of this Manual, which was based on collection of pesticides in ethylene glycol.

REFERENCES:

1. Direct Chromatographic Determination of Carbamate Pesticides Using Carbowax 20M-Modified Supports and the Electrolytic Conductivity Detector, Hall, R. C., and Harris, E. E., J. Chromatogr. 169, 245-259 (1979).

2. Analysis of Pesticide Residues by Chemical Derivatization. II. N-Methylcarbamates in Natural Water and Soils, Coburn, J. A., Ripley, B. D., and Chau, A. S. Y., J. Assoc. Off. Anal. Chem. 59, 188-196 (1976).

3. Sources of Emissions of PCPs into the Ambient Atmosphere and Indoor Air (EPA-600/4-78-022, March 1979), MacLeod, K. E., Analytical Chemistry Branch, U. S. EPA, ETD, HERL, Research Triangle Park, NC.

4. Separation of PCBs, Chlordane, and pp'-DDT from Toxaphene by Silicic Acid Column Chromatograp-y, Bidleman, T. F., Matthews, J. R., Olney, C. E., and Rice, C. P., J. Assoc. Off. Anal. Chem., 61, 820-828.

5. A One-Step Method for the Determination of Carbamate Pesticides by Derivatization with α-Bromo-2,3,4,5,6-Pentafluorotoluene (EPA-600/4-79-036, September, 1979), Jackson, M. D., Soileau, S. D., Sovocool, G. W., and Sachleben, S., Analytical Chemistry Branch, U. S. EPA, ETD, HERL, Research Triangle Park, NC.

6. Evaluation of a Commercial Instrument for Chlordane and Heptachlor Sampling (USAF-75M-12, August, 1975), Thomas, T. C., and Jackson, J. W., Environmental Health Laboratory, McClellan AFB, CA.

(See also references listed in Section 8,A)

II. PRINCIPLE:

Sampling media are Soxhlet extracted with hexane-diethyl ether (95:5 v/v). Chlorinated pesticides and PCPs are measured by EC GLC after column chromatographic cleanup on alumina. PCBs are separated from technical chlordane and other pesticides by column chromatography on silicic acid deactivated with 3% distilled water.

III. EQUIPMENT:

1. Gas chromatograph, Tracor 222 or 560, equipped with linearized ^{63}Ni FPD, and electrolytic conductivity detectors, or equivalent.

2. Rotary vacuum evaporator, e.g., Büchii, with 250, 500, and 1000 ml round bottom flasks.

3. Centrifuge tubes, 15 ml, graduated.

4. N-evap apparatus for evaporation of solvent under a gentle nitrogen stream, Organomation Corp., Northborough, MA, with a 40°C water bath.

5. Extractor, Soxhlet, 1000, 500, and 250 ml.

6. Separatory funnel, 500 ml.

7. Buchner filtration apparatus.

8. Cleanup microcolumn, 10 cm x 5 mm i.d. disposable pipet or Chromaflex column, size 22, 20 cm x 7 mm, Kontes, Vineland, NJ, K 420100-0022.

9. Chromatoflo chromatography column, 25 cm x 9 mm i.d., Pierce # 29020, equipped with a Teflon mesh support membrane, Pierce # 29268, lower end plate, adapter, and 500 ml solvent reservoir (Ace # 5824-10).

IV. REAGENTS:

1. Solvents, glass distilled, pesticide quality, or equivalent.

2. Diethyl ether, analytical reagent grade, Mallinckrodt # 0850, containing 2% ethanol.

3. Pesticide standards and commercial PCB mixtures, 98-100% pure, obtainable from the Pesticide Repository, U. S. EPA, ETD, HERL, Research Triangle Park, NC (MD-69).

4. Individual PCBs, obtainable from RFR Corp., Hope, RI.

5. Alumina, basic, 60 mesh, Alfa Products. Adjust to Brockmann activity IV by adding 6% (w/w) distilled water to the adsorbent in a flask, stoppering, and shaking well; allow to equilibrate for at least 15 hours before use. Discard after two weeks.

6. Sylon CT (dimethyldichlorosilane in toluene), Supelco, Bellefonte, PA.

7. Potassium hydroxide, analytical reagent grade; prepare hydrolysis solution by dissolving 10 grams in 100 ml of methanol in a low actinic flask; discard when discoloration first appears.

8. Sulfuric acid, analytical reagent grade, 50% aqueous solution.

9. Sodium sulfate, analytical reagent grade, Soxhlet extracted with pesticide grade benzene and oven dried before use.

10. Potassium carbonate, analytical reagent grade.

11. Pentafluorobenzyl (PFB) bromide reagent, 1% (v/v); prepare by dissolving 1 ml of reagent (Pierce Chemical Co., Rockford, IL, No. 58220) or α-bromo-2,3,4,5,6-pentafluorotoluene (Aldrich Chemical Co., Milwaukee, WI) in 100 ml of acetone in a low actinic volumetric flask. Prepare fresh every 2-3 weeks. Caution: the reagent is a strong lachrymator!

12. Nitrogen gas, dry, purified.

13. Silica gel, grade 950, Davison Chemical Co., Baltimore, MD, deactivated by adding 1.5% (w/w) distilled water and mixing for 2 hours. Store in a tightly stoppered container in a desiccator.

14. Silicic acid, Mallinckrodt AR, 100 mesh; heat at 130°C for at least 7 hours and cool to room temperature in a desiccator; to deactivate, weigh into a bottle, add 3% (w/w) distilled water, seal tightly, shake well, and place in a desiccator for at least 15 hours. Discard any adsorbent not used within one week.

V. EXTRACTION OF SAMPLING MODULE:

1. Place the sampling medium (Section 8,A) in a Soxhlet extractor, handling with forceps rather than hands.

   NOTE: After sampling, the glass fiber filters and foam plugs should have been wrapped in aluminum foil until analysis. Use plugs and filters carried to the field along with those employed for sampling as controls.

2. Extract with an appropriate volume of *n*-hexane-acetone-diethyl ether (47:47:6 v/v) for 16-24 hours at 4 cycles per hour for the large Soxhlets and 8-12 hours at 8 cycles per hour for the smaller Soxhlets.

   NOTE: As examples, extract large foam plugs in 1000 ml Soxhlet extractors with a total of 300-750 ml of solvent, and smaller plugs and filters in 500 ml Soxhlets with 200-350 ml.

3. Remove the boiling flask to a rotary evaporator and reduce the solvent volume to approximately 5 ml.

4. Transfer the concentrate to a 15 ml graduated centrifuge tube with rinsing.

VI. DETERMINATION OF OCl PESTICIDES AND PCBs:

1. Reduce the volume in the 15 ml tube to below 1 ml by careful evaporation under a gentle stream of nitrogen at room temperature.

2. Carry out alumina cleanup as follows:

   a. Place a small plug of preextracted glass wool in the Chromaflex column and wash with 10 ml of hexane.

   b. Pack the column with 10 cm of activity grade IV alumina.

   c. Transfer the sample from the centrifuge tube to the top of the column; rinse the tube three times with 1 ml portions of *n*-hexane, adding each rinse to the column.

   d. Elute the column at a rate of ca 0.5 ml per minute with 10 ml of *n*-hexane, collecting the eluate in a 15 ml centrifuge tube.

   e. Adjust the final volume of the eluate to 10 ml for gas chromatographic analysis.

3. When necessary, separate PCBs from technical chlordane by silicic acid chromatography as follows:

   a. Place 3 grams of deactivated silicic acid in a Chromatoflo column assembly.

   b. Wash the column with hexane.

   c. Place the sample, concentrated to less than 1 ml, on the column and add 130 ml of hexane to the reservoir.

   d. Apply nitrogen pressure to the column to increase the flow rate to ca 1 ml/minute.

   e. Collect the eluate in three fractions: Fraction I (0-30 ml) contains all the HCB and Aroclor 1254 and most of the Aroclor 1242; Fraction II (31-50 ml) contains the remainder of Aroclor 1242, *p,p*'-DDE, some of the *o,p*'-DDT and toxaphene, and the early eluting peaks of technical chlordane; Fraction III (51-130 ml) contains the remainder of the technical chlordane, including all of the *cis*- and *trans*-chlordane *p,p*'-DDT, and 30% of the toxaphene.

   f. Elute dieldrin, *p,p*'-DDD, 6% of the toxaphene, and the remaining pesticides with 15 ml of dichloromethane.

   g. Adjust the fraction volumes and analyze by GLC.

4. Blank values of unused plugs determined by extraction and alumina cleanup of the extract should be equivalent to < 1 pg/m^3.

VII. DETERMINATION OF OP PESTICIDES:

1. Adjust the final volume in the centrifuge tube as required.

2. Inject directly without cleanup into the gas chromatograph equipped with an FPD detector.

   NOTE: OP compounds are retained by the alumina column, necessitating their analysis without this cleanup.

VIII. DETERMINATION OF CARBAMATE PESTICIDES:

1. Adjust the final volume in the centrifuge tube as required.

2. Inject directly into the gas chromatograph containing a 3% OV-101/Ultra Bond 20M column and electrolytic conductivity detector.

3. As an alternative to direct analysis, determine carbamates by EC GLC after chemical derivatization with α-bromo-2,3,4,5,6-pentafluorotoluene as follows:

   a. Exchange the 5 ml of solvent in the concentrated extract in the rotary evaporator flask (Subsection V,3) for methylene chloride by careful evaporation just to dryness and redissolving of the residue.

b. Add 2 ml of 10% (w/v) methanolic potassium hydroxide to the methylene chloride solution and hydrolyze at room temperature overnight.

NOTE: Hydrolyze and derivatize a mixture of standard carbamates of interest in exactly the same way in parallel with samples.

c. Transfer the hydrolysis solution, by washing with 50-60 ml of distilled water, into a 500 ml separatory funnel and add 50 ml of methylene chloride.

d. Shake briefly and discard the methylene chloride.

e. Acidify to pH ≤ 2 with ca 0.3-0.5 ml of 50% sulfuric acid.

f. Extract the hydrolysis solution with two 50 ml portions of benzene, and dry the benzene by suction filtration through a 10 gram sodium sulfate column into a 250 ml round bottom flask.

g. Evaporate the benzene to 1-2 ml on a rotary evaporator with a water bath at 40°C.

h. Transfer to a 15 ml centrifuge tube by rinsing with 5-6 ml of acetone.

i. Add 25 μl of 5% aqueous potassium carbonate and 100 μl of 1% PFB bromide solution to the centrifuge tube.

j. Stopper, shake, and react at room temperature for at least 3 hours or at 60°C for 30 minutes (loosely stoppered if heated).

NOTE: See Miscellaneous Note 1 for a similar derivatization procedure combining hydrolysis and derivatization into one step.

4. Cleanup and fractionation of carbamate derivatives:

a. Add 2 ml of isooctane to the derivatized solution and evaporate to 1 ml in a 35-40°C water bath with dry nitrogen gas.

b. Repeat the isooctane addition and evaporation to 1 ml.

c. Prepare a cleanup micro column by adding 1 gram of deactivated silica gel to a disposable pipet or Chromaflex column.

d. Prewet the column with 5 ml of hexane, and place the isooctane solution containing the derivatives into the column when the level of the wash liquid just reaches top of the bed.

e. Wash the centrifuge tube with 1 ml of hexane and add this solution to the column.

f. Wash the column with 5 ml of hexane-benzene (95:5 v/v). This fraction containing excess reagent is discarded.

g. Next elute the column in turn with 6 ml of hexane-benzene (75:25 v/v) (Fraction I), 8 ml of hexane-benzene (25:75 v/v) (Fraction II), and 10 ml of pure benzene (Fraction III), collecting each eluate in a clean centrifuge tube. Each eluent is added after the previous one has just reached the top level of the column.

NOTE: Determine the elution pattern of the PFB ether derivatives of the carbamates of interest on the silica gel column under local laboratory conditions. The compounds studied by Coburn et al. eluted as follows:

PFB ether derivative	Recovery, %[a]		
	Fraction I	Fraction II	Fraction III
Propoxur	--	93-97	2-5
Carbofuran	--	94-97	2-4
3-Ketocarbofuran	--	--	96-98
Metmercapturon	84-89	12-15	--
Carbaryl	97-100	0-2	--
Mobam	96-99	0-3	--

---

[a] Obtained by comparing the peak areas of 5 samples passed through the silica gel columns with 3 samples not fractionated.

h. Concentrate the eluate fractions as needed and analyze by EC GLC.

IX. GAS CHROMATOGRAPHY:

1. Determine OCl and OP pesticides on a 183 cm x 4 mm i.d. glass column packed with 1.5% OV-17/1.95% OV-210 and/or 4% SE-30/6% OV-210 on 80-100 mesh Gas Chrom Q; column, 200°C; injection port, 215°C; nitrogen carrier gas, 60-85 ml/minute; electron capture detector for OCl pesticides and P-mode FPD (200°C) for OP pesticides.

2. Determine PCBs by EC GLC under the above conditions on a similar column packed with 3% OV-1 on Gas Chrom Q at 180°C. Alternatively, use columns containing 3% OV-225 on Supelcoport, 80-100 mesh or 4% SE-30/6% OV-210 on Gas Chrom Q, 100-200 mesh at 200°C.

3. Determine carbamates on a 103 cm x 2 mm i.c. silanized (Sylon CT) glass column packed with 3% OV-101 on Ultra Bond 20M (RGC 005, RFR Corp., Hope, RI) [Section 4,A(7)]; column and injection port, 170-185°C; helium carrier gas, 25 ml/minute; N-mode Hall electrolytic conductivity detector (Section 4,C): reductive mode with nickel wire catalyst and strontium hydroxide scrubber; conductivity solvent, water-isopropanol (85:15 v/v); hydrogen reaction gas, flow rate 80 ml/minute; furnace temperature, 720°C; inlet temperature, 10°C above the column temperature; transfer line, 200°C.

4. Determine carbamate PFB ether derivatives by EC GLC on a 183 cm x 4 mm i.d. glass column containing 3% (w/w) OV-225 on 80-100 mesh Chromosorb W (HP). An alternate column for Fractions II and III of the silica gel cleanup column is 3.6% (w/w) OV-101/5.5% (w/w) OV-210 on acid washed, dimethyldichlorosilane treated Chromosorb W. Use the following operating conditions: injector temperature 205°C, column 190°C, detector 280°C; 5% methane-argon carrier gas flow rate 50 ml/minute + 20 ml/minute purge for the OV-101/OV-210 column, 30 ml/minute + 20 ml/minute purge for OV-225; EC detector in pulsed mode with electrometer settings of 55 V, 90 μsec pulse rate, 8 μsec pulse width, and 6.4-1.6 x 10^{-9} amp full scale attentuation. Relative retention times of PFB ether derivatives on the two columns are as follows:

Derivative	RRT OV-101/OV-210 [a]	RRT OV-225 [b]
Propozur	0.43	0.41
Carbofuran	0.64	0.63
3-Ketocarbofuran	1.15	1.13
Metmercapturon	1.26	1.28
Carbaryl	1.38	1.31
Mobam	1.48	1.31

---

[a] Relative to aldrin 2.7 minutes

[b] Relative to aldrin 7.9 minutes

Quantitate by comparison of peak areas against chromatograms of derivatized standard carbamate phenols. The standard derivatives are synthesized as follows:

a. React each carbamate phenol with a 10-fold molar excess of PFB bromide in acetone and a 10-fold molar excess of methanolic KOH.

b. Reflux for 2 to 3 hours, cool, and remove the solvent on rotary evaporator.

c. Dissolve the product in benzene and wash the benzene twice with equal volumes of 0.1 M K_2CO_3.

d. Dry the benzene, using suction, by passing it through a 10-20 gram column of anhydrous Na_2SO_4.

e. Remove the benzene on a rotary evaporator and recrystallize from hexane or methanol.

5. Inject 5 μl or another appropriate volume of the sample extract or cleanup column eluate into the gas chromatograph.

6. Record chromatograms under the above parameters and measure retention times relative to aldrin or another suitable reference standard.

7. Compare the relative retention time of each component of interest against those of the corresponding primary standard.

8. Quantitate peaks in the usual way, i.e., by measuring peak heights to the nearest mm when the base width is <1 cm or via peak areas by integration or triangulation for broader peaks.

9. Confirm results as required by combined GLC/MS or some other appropriate procedure (EPA Pesticide Analytical Quality Control Manual, Chapter 8).

10. Commercial PCB mixtures are quantitated by comparisons of the total heights or areas of GLC peaks with the corresponding peaks in the standard used. The absolute retention times on the 3% OV-1 column for the peaks used were as follows:

    Aroclor 1242 - 2.39, 2.65, 3.11, 3.33, 3.94, 4.37, 4.67, 5.59, and 6.25 minutes.

    Aroclor 1254 - 3.81, 4.28, 4.61, 5.55, 6.68, 7.76, 8.23, 9.83, 11.47, and 13.67 minutes.

    With the SE-30/OV-210 column, the total peak heights of the peaks shown in Figure 1 can be used for quantitation.

    Make Aroclor standards by dissolving the Aroclor in isooctane, and prepare dilutions in hexane. Store stock solutions in brown bottles at -10°C. Remake working standards periodically from these and store in a refrigerator when not in use.

X. RECOVERY DATA:

Measurements made with six polychlorinated biphenyls using the dual sorbent trap (Section 8,A) are shown in Table 1. The dichlorobiphenyl and one of the two trichlorobiphenyls tested appeared to be more efficiently collected by all of the dual traps than by the trap containing only PUF. Preferential vaporization of the more volatile PCBs from the fortified felt pad (trace B, Figure 2) and more efficient trapping of the less volatile PCBs by the PUF alone were found to occur. Studies completed to date with a variety of pesticides have been less conclusive. Essentially no differences were observed between the sorbent systems for the eleven pesticides shown in Table 2. Work is yet to be completed with α- and γ-BHC and several carbamates.

The ERCO high volume sampler has been shown to be efficient for malathion collection and has been evaluated for heptachlor and chlordane, methyl parathion from treated foliar survaces, diazinon vapors, chlorpyrifos, and the carbamate propozur (Table 3). In all cases, collection efficiencies were determined over only short sampling periods (2-4 hours). Due to the thinness of the filter pads (3 mm), significantly longer sampling periods would be expected to lead to potential breakthrough of sorbed vapors. Earlier collection efficiency data for separate mixtures of OCl and OP pesticides by volatilization from a wool felt filter into a tandem pair of foam plugs is shown in Tables 4 and 5.

The retentions of Aroclor 1242 and Aroclor 1254 on PUF after 4 hours at 4 liters/minute air flow are given in Table 6. This determination was made by injecting the PCB mixtures in hexane solution into the foam, allowing it to air dry at ambient temperatures for one hour, then placing it into module b (Section 8,A, Figure 3) and pulling prefiltered air through it into a second PUF trap of the same dimensions. Both traps were extracted after 4 hours to determine the amount of PCBs remaining in the first and that displaced to the second trap. As expected, retentions were better than those found for the same type PUF when exposed to 24 hours of air flow at 225 liters/minute in the high volume EPA sampler.

Collection efficiencies were measured by vaporizing known quantities of the test compounds or mixtures into the PUF. These data are presented in Table 7, along with comparative values for the high volume EPA sampler. Again, the lower flow rates and shorter sampling times appear to favor the low volume sampler. Gas chromatograms in Figure 3 show the selective volatilization of the lower boiling components of technical chlordane from the vapor generator and the nonuniform trapping of those vapors by the foam.

The efficiency of the recovery process was checked by spiking foam plugs with Aroclor standards and then carrying them through the sample extraction and cleanup procedure. A syringe or pipet was used to fortify the small plugs with a known amount of Arochlor 1242 and Aroclor 1254 in hexane. After allowing the hexane to evaporate, the plugs were extracted in the usual manner and the extracts were concentrated, run through alumina, and analyzed. Four small fortified plugs gave 77% ± 11% recovery for the Aroclor 1242 and 100% ± 18% for Aroclor 1254 when spiked with 100 ng of each of these standards.

In summary, polyurethane foam in both high and low volume air samplers, used in conjunction with the analytical procedures described in this section, serves well for the determination of low levels of many PCBs and relatively nonvolatile pesticides in indoor and outdoor air. The use of tandem traps does not always improve collection efficiencies, despite this expectation.

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In summary, polyurethane foam in both high and low volume air samplers, used in conjunction with the analytical procedures described in this section, serves well for the determination of low levels of many PCBs and relatively nonvolatile pesticides in indoor and outdoor air. The use of tandem traps does not always improve collection efficiencies, despite this expectation.

IX. MISCELLANEOUS NOTE:

An alternate one-step method for the determination of carbamate pesticides by derivatization with α-bromo-2,3,4,5,6-pentafluorotoluene has been devised by M. D. Jackson *et al*. (Reference 5). The procedure, which combines the alkaline hydrolysis and derivatization steps, was tested on 23 carbamate pesticide standards, 18 of which formed gas chromatographable derivatives using the standard EPA GLC parameters. Selected products were studied by GLC MS, which indicated that those carbamates hydrolyzing to give phenolic intermediates formed derivatives with one fluorine on the PFB ring displaced by an ethozide ion via aromatic nucleophilic substitution. The derivatization procedure and GLC relative retention and sensitivity values follow: Details of methods and further results (Linearity of EC response, quantity of derivative to give 50% FSD, storage of derivatives, background interferences, mass spectra) are given in the complete report available from the above address.

Procedure

a. Pipet one ml of alcoholic potassium hydroxide, 0.1 ml of derivatizing reagent, and one ml of carbamate standard into a 15 ml culture tube with a Teflon lined screw cap.

b. Place the culture tube in a preheated (95 ±1°C) tube block heater for two hours.

NOTE: The length of time and temperature are critical, for overheating can cause an increase in the formation of extraneous gas chromatographic peaks.

c. Remove, allow to cool at room temperature, and add 5 ml of distilled water and 4 ml of *n*-hexane to the culture tube.

d. Place the culture tube on a tube rotator (60 rpm) for two minutes and, at the end of this time, transfer the *n*-hexane layer to a 15 ml centrifuge tube.

e. Add an additional 4 ml of *n*-hexane to the culture tube, and place in the tube rotator for an additional two minutes.

f. Combine the *n*-hexane layer with the previous *n*-hexane extract.

g. Bring the final volume of the centrifuge tube to 10 ml with *n*-hexane. The sample is now ready for further cleanup or gas chromatographic analysis.

GLC Results - See Table 8

TABLE 1. HIGH-VOLUME COLLECTION EFFICIENCIES OF PCB CONGENERS ON FOAM/GRANULAR SORBENT COMBINATIONS

PCB	Calc Air Conc. (ng/m3)	% Collection on Foam/Sorbent Combinations After 24 hr. at 225 L/min.					
		Foam Alone	Chromo 102 (20/40)	Porapak R (50/80)	XAD-2 (16/50)	Tenax GC (60/80)	Florisil (16/30)
4,4'-di	2-20	62	82	82	96	85	111
2,4,5-tri	0.2-2	36	80	87	91	-	92
2,4',5-tri	0.2-2	86	81	89	93	80	88
2,2',5,5'-tetra	0.2-2	94	81	88	88	81	92
2,2',4,5,5'-penta	0.2-2	92	79	92	96	84	97
2,2',4,4',5,5'-hexa	0.2-2	86	84	92	95	85	93

TABLE 2. HIGH-VOLUME COLLECTION EFFICIENCIES OF PESTICIDES ON FOAM/GRANULAR SORBENT COMBINATIONS

Pesticide	Calc Air Conc. (ng/m3)	% Collection on Foam/Sorbent Combinations After 24 hr. at 225 L/min.					
		Foam Alone	Chrom 102 (20/40)	Porapak R (50/80)	XAD-2 (16/20)	Tenax GC (60/80)	Florisil (16/30)
Aldrin	0.3-3.0	28	34	35	33	-	40
p,p'-DDE	0.6-6.0	89	83	93	135	71	138
p,p'-DDT	1.8-18.0	83	77	89	138	69	119
Mirex	1.8-18.0	93	94	95	132	78	123
Tech. Chlordane	15-150	73	85	74	87	73	97
α-Chlordane	1.5-15.0	114	108	96	102	100	98
γ-Chlordane	1.5-15.0	126	104	91	96	93	100
Diazinon	3.0-30.0	63	72	59	71	76	72
Methyl Parathion	1.8-18.0	91	82	72	80	87	83
Ethyl Parathion	3.6-36	96	85	72	81	86	83
Malathion	0.9-9.0	97	88	78	89	91	81

TABLE 3. ERCO SAMPLER COLLECTION EFFICIENCIES AT 183 L/MIN. FOR 2 HRS.

Compound	Calc Air Conc. (μg/m3)	Collection Efficiency (%)
Methyl Parathion	0.02-150	105
Diazinon	0.11-2.2	93
Chlorpyrifos	0.02-0.22	77
Propoxur	4.5	54
Chlordane	2.9-5.5	69-72*
Heptachlor	0.8-4.0	66-98*

*At 145-170 L/min. for 2 to 4 hrs. (Thomas & Jackson)

TABLE 4. COLLECTION EFFICIENCIES OF POLYURETHANE FOAM AT 225 L/MIN FOR CHLORINATED PESTICIDES VS AIR CONCENTRATIONS

	Calc. Air Conc. (ng/m^3)	Efficiency (%)	Statistical Data	
			n	σ
γ-BHC	0.15	53.2	6	6.4
	0.08	38.4	5	14.0
	0.03	55.0	6	20.7
Aldrin	1.50	58.5	2	2.1
	0.30	35.3	9	24.1
	0.06	50.2	5	9.4
p,*p*'-DDE	3.10	95.7	3	9.0
	0.60	96.2	7	16.4
	0.30	104.8	7	24.5
	0.10	101.0	3	27.6
p,*p*'-DDT	9.20	114.7	3	12.6
	1.84	94.6	8	13.4
	0.92	93.6	7	9.0
	0.37	83.0	2	21.2
Mirex	9.20	103.7	3	11.6
	0.60	100.4	7	7.1
	0.30	98.7	6	5.6
	0.12	105.4	5	5.7

TABLE 5. AVERAGE COLLECTION EFFICIENCIES OF POLYURETHANE FOAM FOR ORGANOPHOSPHORUS PESTICIDES AT 225 L/MIN AND 184 L/MIN

Pesticide	Air Volume (m^3)	Calc. Air Conc. (ng/m^3)	% Collected	Statistical Data n	Statistical Data σ
Diazinon	326	30.7	70.4	5	3.97
	265	18.9	91.0	6	16.19
	265	3.8	75.5	6	14.40
Methyl parathion	326	18.4	73.6	5	4.56
	265	11.3	73.3	5	6.52
	265	2.3	71.9	4	4.12
Malathion	326	36.8	87.2	5	33.40
	265	22.6	76.6	5	12.47
	265	4.5	81.2	4	14.68
Parathion	326	9.2	84.8	5	4.15
	265	5.7	70.3	5	4.70
	265	1.1	65.8	4	3.75

TABLE 6. RETENTION OF PCB MIXTURES ON POLYURETHANE FOAM

PCB Mixture	Initial Quantity (μg)	% of Original Recovered After 4 hr. at 4 L/min 1st Plug*	2nd Plug	Total
Aroclor 1242	2	87	9	96
Aroclor 1254	2	99	0.4	99

*Into which initial quantity was injected.

TABLE 7. COMPARISON OF COLLECTION EFFICIENCIES OF POLYURETHANE FOAM IN LOW- AND HIGH-VOLUME SAMPLES

Compound or Mixture*	% Collected After: 4 hr. at 2.5 L/min	24 hr. at 225 L/min
Aroclor 1242	99	76
Aroclor 1254	90	85
Aroclor 1260	117	100
tech. Chlordane	84	73
α-Chlordane	108	114
γ-Chlordane	101	126

* Introduced as vapors. Calculated air concentrations for duration of sampling periods were 1 to 2 μg/m^3 for the low-volume sampler and 0.01 to 0.02 μg/m^3 for the high-volume sampler.

TABLE 8. GAS CHROMATOGRAPHY OF CARBAMATE PESTICIDE PFBB DERIVATIVES

Pesticide	Relative Retention Time[a] 1	Relative Retention Time[a] 2	Derivatization Linearity, μg[b]	Minimum Detection level, ng[c] 1	Minimum Detection level, ng[c] 2
Aldicarb	0.56	0.60	1-100	0.080	0.040
Aminocarb	2.0	1.9	1-100	1.6	0.49
Barban	2.0	3.0	100-1000	20	33
Benthiocarb	0.91	1.00	10-1000	0.65	0.48
Carbaryl	4.7	3.6	1-1000	0.20	0.18
Carbofuran	2.1	d	2-1000	0.70	d
CDEC	0.64	0.62	0.1-1000	0.024	0.024
Chloropropham	0.39[e], 2.9	0.40[e], 3.0	10-1000	0.72	0.72
Dismedipham	2.8	1.8	100-1000	6.3	8.4
Formetanate·HCl	1.7	2.2	100-1000	4.7	4.1
Karbutilate	2.5	2.1	100-1000	9.7	9.9
Meobal	1.2	1.0, 1.2[e]	10-1000[f]	0.091	0.096
Methiocarb	3.7	3.1	1-1000	0.35	0.30
Methomyl	0.37, 0.71 1.3[e]	0.25, 0.40 1.5[e]	1-100	0.031	0.026
Penmedipham	1.8, 2.3[e] 2.8	1.5, 1.6[e] 1.8	100-1000	18	14
Promecarb	1.4	1.4	10-1000[f]	0.14	0.14
Propoxur	1.2	1.2	10-1000	0.080	0.081
Thiophanate methyl	3.0	3.3	10-100[f]	0.0087	0.012

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[a] Relative to aldrin (1.0) on (1) 1.5% OV-17/1.95% OV-210 or (2) 4% SE-30/6% OV-210 on 80-100 mesh Gas Chrom Q at 220°C

[b] Checked over a concentration range from 0.1-1000 ng/μl, if possible, or to the limits of detection; allowances of › 15% were tolerated in determining the linearity range.

[c] 10% full scale deflection with ^{63}Ni EC detector at 350°C, nitrogen carrier gas, 100 ml/minute, columns (1) and (2) as in [a]

[d] Characterization was not attempted on the SE-30/OV-210 column due to high background interference

[e] Major peak

[f] Linearity could not be confirmed below 10 μg due to blank peak interference

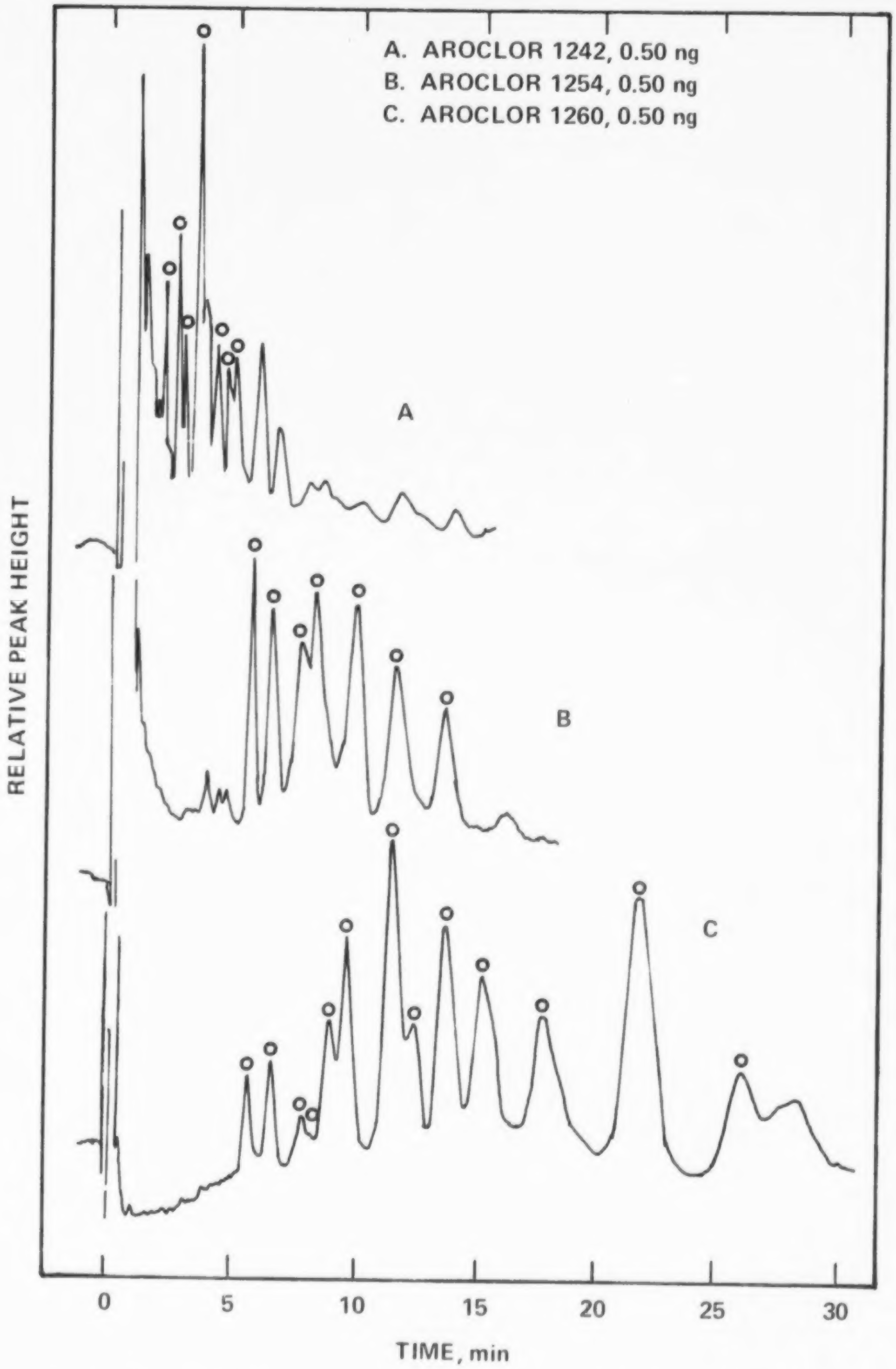

Figure 1. Chromatograms showing peaks used in quantifying PCB, SE-30/OV-210.

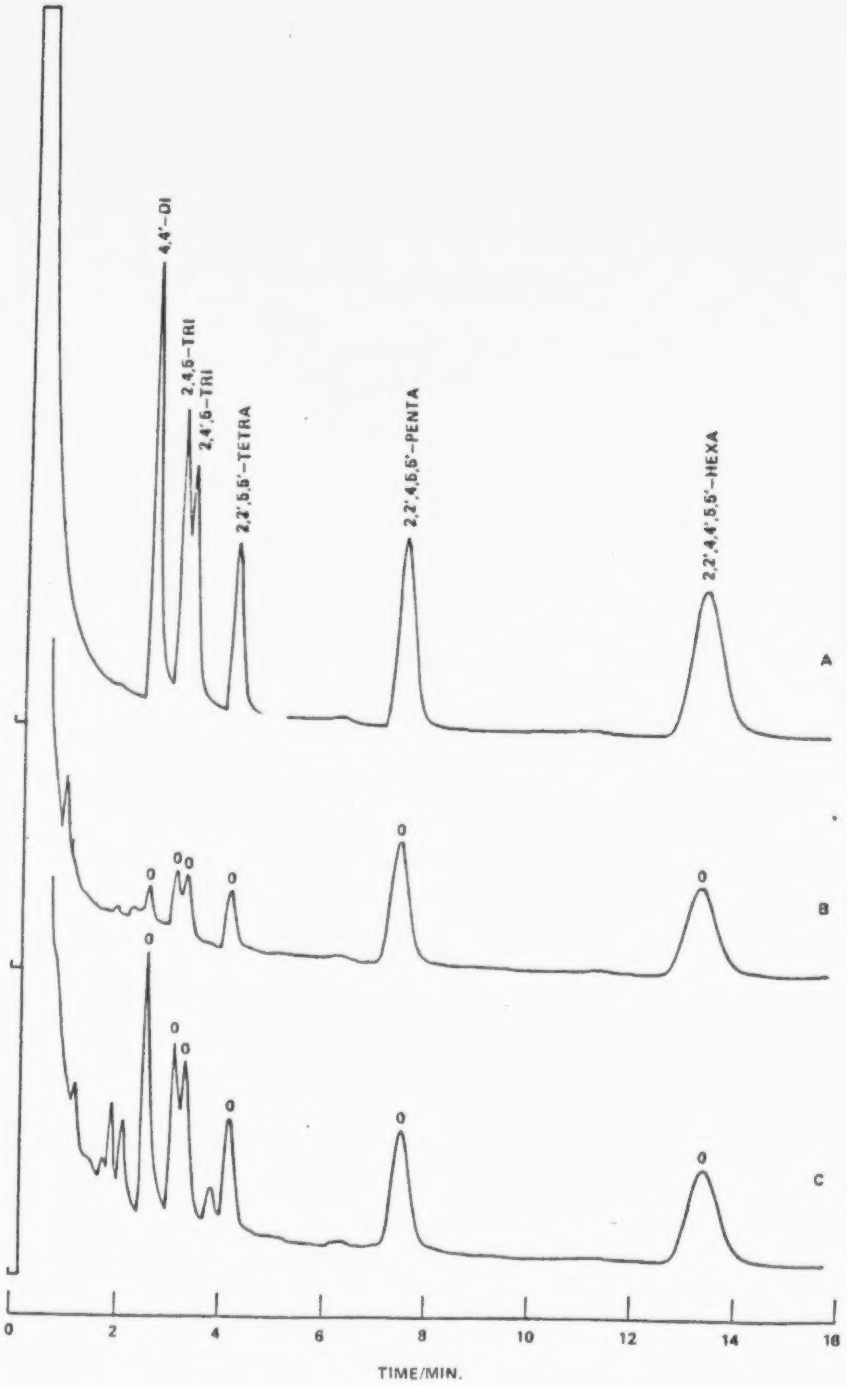

Figure 2. Electron capture gas chromatograms showing collection of polychlorinated biphenyls or polyurethane foam at 225 L/min. Trace A is of the mixture of PCB congeners in n-hexane, B is of the residue remaining on the wool felt pad after 24 hr (at one-half the attenuation) and C is the residue collected by the foam.

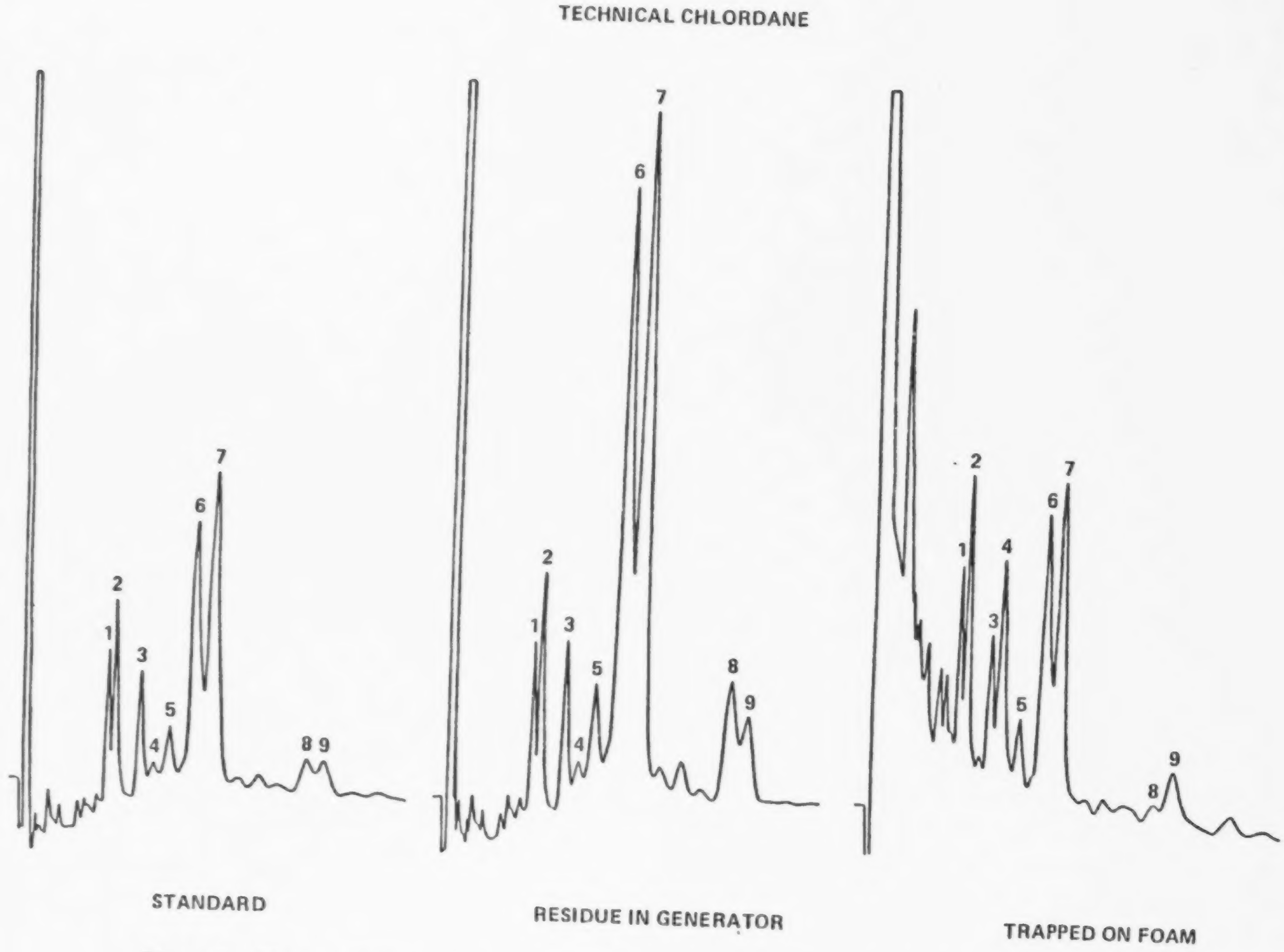

Figure 3. Electron capture gas chromatograms showing selective vaporization of technical chlordane and non-uniform trapping efficiencies of the components on polyurethane foam (after 4 hrs at 4 L/min).

POLYCHLORINATED BIPHENYLS

INTRODUCTION

All chromatographers with experience in the analysis of biological materials are only too familiar with problems involving "artifact" peaks which, based on their retention characteristics, could be identified as aldrin, dieldrin, heptachlor, DDT or one of its metabolites and/or some other common pesticides. Several years ago one series of compounds came to light as a contributory source of a great deal of this confusion. These were the polychlorinated biphenyls. The first report of detection in the environment came from Sweden in 1966, and a year later from the United States, despite the fact the materials have been used for 40 years.

The prime manufacturer in the United States of these products is the Monsanto Chemical Company. A series of PCB's have been marketed under the trade name of Aroclor. A company bulletin listed many products in which the materials could be used as plasticizers, flame retardants, insulating fluids, or to impart some other useful quality. Among these products were natural and synthetic rubber, electrical products, floor tile, printer's ink, coatings for paper and fabric, brake linings, auto body sealants, paints, varnishes, waxes, asphalt, and many adhesives and resins. The PCB's were at one time recommended by Monsanto for mixing with chlorinated insecticides to suppress their vaporization and extend their persistence. At the time of this current printing we understand that only two of the Aroclor compounds are being produced.

Since the first U. S. environmental detection of the PCB's in peregrine falcon eggs in 1967, their presence has been reported in many segments of the environment. To mention a few, gull eggs in San Francisco Bay, human milk in Colorado, human adipose tissues from many parts of the U. S., fish from several areas, fresh and saltwater.

We are presenting some information on the PCB's in this manual primarily to alert chromatographers to the ever potential presence of these contaminants in routine samples, particularly of adipose tissue. A method has been reported by Amour and Burke for separating and PCB's from the common chlorinated pesticides. A reprint of this method is included in Section 9,C. Typical chromatograms have been obtained for five Aroclors, along with numerical data for relative retention and response on the two working columns of the program. These data are presented in Subsections 9,E and 9,F.

Manipulation of the analytical procedures for PCB's is somewhat more difficult than that of a number of the other methods in this manual. However, competent residue chemists should experience no sustained difficulty in coping with the procedures.

DETERMINATION OF PCBs IN HUMAN MILK

MACRO METHOD

I. INTRODUCTION:

The analytical procedure described in this section was modified and used in a survey, conducted by the Colorado Epidemiologic Pesticides Studies Laboratory, Colorado State University for measuring the levels of PCBs in mother's milk. The survey was made under a contract that the University had with the EPA Office of Pesticide Programs. The method was evaluated in the EPA Analytical Chemistry Branch, ETD, HERL, Research Triangle Park, and found to be satisfactory for obtaining an approximation of PCB levels. It should be stressed that the method, in common with other existing PCB analytical procedures, provides only a semi-quantitative approximation for fingerprinting the multicomponent family of isomeric PCB compounds and is not absolutely quantitative. The approximation levels of PCBs obtained from the method must be interpreted with great care. Section 9,B,(2) presents alternative micro methods for analysis of mother's milk as well as confirmation procedures for the PCBs.

REFERENCES:

1. Residues of Organochlorine Pesticides and Polychlorinated Biphenyls and Autopsy Data for Bald Eagles, 1971-72, Chromartie, E., et al., Pestic. Monit. J. 9, 11 (1975).
2. Method for Separating PCB's from DDT and its Analogs, Armour, J. A., and Burke, J. A., J. Assoc. Offic. Anal. Chem. 53, 761 (1970); see also Section 9,C.

II. PRINCIPLE:

The procedure consists of isolating the fat from the milk, extracting PCBs from the fat, cleanup of the extract, and electron capture GLC for determination. A weighed milk sample is extracted with acetone and hexane, PCBs are transferred to the hexane layer by adding sodium sulfate solution, and the hexane is dried by passage through a sodium sulfate column. Part of the sample is used for a lipid determination, and the rest is partitioned with acetonitrile and then taken through a Florisil column fractionation. Identification and quantification are done by GLC using an electron capture detector and two columns with different resolution characteristics. Further confirmation of PCBs and pesticides can be obtained by GLC with electrolytic conductivity detector (Cl mode) and GLC MC of pooled samples.

III. SAMPLE COLLECTION:

1. Samples are manually expressed by participants into glass tubes equipped with plastic screw caps and Teflon liners. The filled tubes are kept frozen (-10°C) until the time of extraction.

2. Pertinent data must be obtained from donors by the hospital nurse doing the sampling, and careful selection of donors must be made by the field epidemiologist in charge of the program to achieve the goals of any survey. Important data include age and geographical location of each donor, urban or rural location of the hospital, and pesticide usage levels in the donor's area.

IV. EQUIPMENT:

1. Gas chromatograph, such as a Tracor 220 or equivalent, equipped with ^{63}Ni or ^{3}H electron capture detector. If desirable for confirmation of residue identity, an electrolytic conductivity detector can be used.

   GLC columns, borosilicate glass, 183 cm x 4 mm i.d., packed with 1.5% OV-17/1.95% OV-210 and 4% SE-30/6% OV-210, both coated on Gas Chrom Q, 80-100 mesh, operated with the specific parameters given under Gas Chromatography, Section X. These packings are available from most gas chromatography supply houses, e.g., Applied Science Laboratories, Inc., Supelco, Inc., etc.

2. Centrifuge bottles, 200 ml, with Teflon-lined screw caps, 3.8 cm diameter.

3. Glass wool, Pyrex, precleaned by rinsing three times with petroleum ether and acetone.

4. Centrifuge, capable of 2000 rpm.

5. Separatory funnels, 125 ml, 500 ml, and 1000 ml.

6. Chromatographic columns, 300 mm x 25 mm o.d., with Teflon stopcocks, with or without fitted glass plates, size 241, Kontes 420530.

7. Flasks, round bottom, short neck, Pyrex, 250 ml and 500 ml.

8. Kuderna-Danish (K-D) evaporative concentrator, 250 ml or 500 ml flask, Kontes 57001; 3-ball Snyder column, Kontes 503000; 1/2 inch steel springs, Kontes 662750; 10 ml graduated concentrator tubes, size 1025, Kontes 570080.

9. Modified micro-Snyder column, 19/22, Kontes K-569251.

10. Glass beads, 3 mm plan.

11. Disposable pipets.

12. Volumetric flasks, 100 ml.

13. Beakers, 50 ml, Griffin.

14. Pipets, 20 ml, class A.

15. Ovens, capable of regulation to 130°C and 37°C.

16. Evaporation apparatus utilizing a nitrogen gas stream.

17. Mixer producing a tumbling action at ca 50 rpm (Fisher Roto-Rack or equivalent).

All glassware is cleaned according to Section 3,A.

V. REAGENTS:

1. Diethyl ether, AR grade, containing 2% ethanol, peroxide free, Mallinckrodt 0850 or equivalent.

2. n-Hexane, acetone, acetonitrile, and petroleum ether of pesticide quality.

3. Eluting mixture, diethylether-hexane (6:94 v/v). Dilute 60 ml of diethyl ether to 1000 ml with hexane. The solution should be kept no longer than 24 hours after preparation.

4. Florisil, 60-100 mesh, PR grade, stored at 130°C until used (see Sections 3,D and 5,A,(1), p. 3 for special comments).

5. Sodium sulfate, anhydrous, reagent grade, granular Mallinckrodt 8024, prewashed or Soxhlet-extracted with hexane prior to use. Prepare sodium sulfate solutions with hexane-extracte deionized water.

6. Silicic acid, SilicAR CC-4 Special, Mallinckrodt 7086, prewashed and activated prior to use as described below in the method.

7. Filter paper, Whatman No. 1, rinsed in hexane. Check the final background and Soxhlet extract the paper if required.

8. PCB and pesticide standards, obtained from the EPA repository, HERL, ETD, Research Triangle Park, NC.

VI. SAMPLE EXTRACTION:

1. Thaw and thoroughly mix the whole milk sample.
2. Weigh 4.5 to 24.3 g into a clean, dry centrifuge bottle.
3. Add enough pre-cleaned glass wool to adhere to the coarse precipitate of the milk solids.
4. Add 100 ml of redistilled acetone to the bottle, shake manually for one minute, and then centrifuge at 1500 rpm for ca 2 minutes.
5. Transfer the acetone to a 500 ml separatory funnel, filtering through Whatman No. 1 filter paper or prerinsed glass wool.
6. Extract the milk precipitate with two 25 ml portions of acetone, shaking but not centrifuging.
7. Combine all three extracts in the 500 ml separatory funnel, following the procedure in step 5.
8. Add 50 ml of hexane to the coarse precipitate of milk solids, shake, centrifuge, decant, and combine with the acetone in the 500 ml separatory funnel. Repeat with 50 ml more of hexane.
9. Add 125 ml of 2% aqueous sodium sulfate solution to the 500 ml separatory funnel.
10. Shake the funnel manually for 1 minute, allow the phases to separate, and discard the lower (aqueous) layer.
11. Repeat steps 9 and 10, again discarding the lower layer.
12. Place 3 inches of sodium sulfate into a size 241 column. Wash the column with 100 ml of hexane and discard the hexane. As the last of the hexane wash just reaches the top of the sodium sulfate, drain the hexane extract from the 500 ml separatory funnel into the column. Allow this extract to sink into the sodium sulfate; then add 100 ml of hexane to the column. Collect all of the eluate in a clean, dry 250 ml concentrator flask.
13. Reduce the volume in the concentrator flask to ca 10 ml and transfer quantitatively, using a clean disposable pipet, to a 100 ml volumetric flask. Dilute to volume with hexane.
14. Pipet a 20 ml aliquot, representing 1/5 of the original milk sample, from the flask into a clean, dry 50 ml beaker. Evaporate the solvent under a nitrogen stream and place the beaker in a 37°C oven overnight for a lipid determination. (Caution: Remove

all hexane before placing beaker in oven.)

NOTE: See Miscellaneous Note 1 for the determination and calculation of lipids.

15. Concentrate the remaining 80 ml of sample and transfer quantitatively to a 125 ml seperator funnel. Adjust the volume of hexane to 15 ml.

VII. LIQUID-LIQUID PARTITIONING:

1. Add 30 ml of acetonitrile, previously saturated with hexane, to the 125 ml separatory funnel and shake vigorously for 2 minutes.

2. After phase separation, draw off the acetonitrile layer into a 1 L separatory funnel containing 550 ml of 2% sodium sulfate solution and 100 ml of hexane.

3. Repeat extraction of the hexane layer in the 125 ml funnel three more times in a similar way, combining all acetonitrile extracts in the 1 L funnel.

4. Stopper the 1 L funnel, invert, vent off pressure, and shake for 2 minutes, releasing pressure periodically as required. Allow the phases to separate and discard the lower (aqueous) phase.

5. Wash the hexane phase with two additional 100 ml portions of 2% sodium sulfate solution, discarding the aqueous washings.

6. Transfer the hexane layer to a K-D evaporator.

7. Attach a 3-ball Snyder column over the evaporator and place in a water bath at 90-100°C. Approximately 4 cm of the concentrator tube should be below the water surface.

8. Concentrate the extract to ca 5 ml, and rinse down the sides of the evaporator and ground glass joint with a total of 3 ml of hexane.

9. Reconcentrate to ca 5 ml under a gentle stream of nitrogen at room temperature.

VIII. FLORISIL COLUMN FRACTIONATION:

1. Prepare a chromatographic column containing 10 cm (after settling) of activated Florisil topped by 4 cm of sodium sulfate. Place a small wad of class wool at the bottom of the column to plug the glass tube and retain the adsorbent.

   NOTE: The small amount of Florisil needed for proper elution should be determined for each different lot by elution of analytical standards.

2. Prewash the column with 100-200 ml of hexane, and discard.

   NOTE: From this point on through the elution process, the solvent level should never be allowed to fall below the top surface of the sodium sulfate layer. If air is introduced into the column, channeling may occur, causing an inefficient column. Each solution is added to the column just as the previous one reaches the top of the bed.

3. Immediately transfer the ca 5 ml of extract from the evaporator tube onto the column, using a 5 ml Mohr pipet or long disposable pipet. Allow the sample to sink into the column.

4. Rinse the evaporator tube with two successive 5 ml portions of petroleum ether, carefully transferring each portion to the column with the pipet.

   NOTE: Delivery of the extract by pipet directly onto the column precludes the need to rinse down the inside column walls.

5. Prepare a complete 500 ml K-D evaporative assembly with 10 ml concentrator tube. Place one glass bead in the tube.

6. Commence elution with 200 ml of the 6% eluting mixture at a rate of 5 ml per minute, collecting the eluate in the K-D assembly. After collection of the fraction, concentrate as in Subsection VII, step 7.

   NOTE: If determination of more polar chlorinated pesticides in addition to PCBs is desired, place a second 500 ml K-D assembly under the column and continue elution with 200 ml of diethyl ether-petroleum ether (15:85 v/v). Pesticides eluting in these fractions are listed in Table 1, Section 5,A,(1).

7. Remove the K-D assembly from the bath and cool to ambient temperature.

8. Disconnect the collection tube from the D-D flask and carefully rinse the joint with a small amount of hexane.

9. Attach a modified micro-Snyder column to the collection tube, place back in the water bath, and concentrate the solution to 1 ml.

   NOTE: If preferred, this concentration can be done at room temperature under a gentle nitrogen stream.

10. Remove the tube from the bath and cool to ambient temperature. Disconnect the tube and rinse the joint with a little hexane.

IX. SILICIC ACID COLUMN FRACTIONATION:

Silicic acid is used for separating PCBs from DDT and some of its analogs also present in the Florisil eluate. The method is a modification by Cromartie et al. (1) of the Armour and Burke (2) procedure, which eliminates use of Celite and air pressure to speed the column elution.

1. Prepare the adsorbent as follows:

   a. Prewash SilicAR CC-4 three times with acetonitrile-hexane-methylene chloride (1:19:80 v/v). Approximately 320 ml of wash solution is used for each 125 g of silicic acid.

   b. Activate the washed adsorbent in a 130°C oven for 24 hours.

   c. Remove the adsorbent from the oven, transfer to a stoppered Erlenmeyer flask, and cool to room temperature.

   d. After cooling, add enough distilled water to a weighed portion of adsorbent to give a 3% deactivated material (3 ml water per 100 g silicic acid). Stopper at once.

   e. Shake the flask for 1 hour on a mechanical shaker, and then allow an additonal 30 minutes of equilibration before use.

2. Pack the column as follows:

   a. Place 20 g of deactivated silicic acid in a beaker and add enough hexane to form a slurry.

b. Pour the slurry into a pre-rinsed size 241 glass column to which 13 mm of anhydrous sodium sulfate has been added.

c. Allow the silicic acid to settle and add 13 mm of sodium sulfate on top to prevent the surface of the acid column from being disturbed when applying the sample.

d. Rinse the column with 50 ml of petroleum ether, discarding the eluate.

3. When the last of the wash just reaches the top of the bed, transfer the sample from the collection tube onto the column using a disposable pipet.

NOTE: Observe the same precaution as in the note under step 2 of the preceding subsection.

4. After the sample has entered the column, elute with 400 ml of petroleum ether at a flow rate of 5 ml per minute.

5. Collect the effluent in two separate fractions. The first 100 ml is Fraction I, in which hexachlorobenzene, mirex, and some other chlorinated pesticides are collected. The remaining 300 ml is Fraction II, which contains PCBs and most of the DDE.

6. Concentrate Fraction II to a suitable definite volume for analysis of PCBs. Fraction I is not analyzed unless it is suspected that some of the PCBs may be eluting in it or if determination of the pesticides in Fraction I is of interest.

NOTE: It is wise to spotcheck Fraction I for the presence of PCBs from time to time. The performance of each batch of silicic acid can be evaluated by elution of standard PCBs and pesticides through a column, as will be done for analyses.

X. GAS CHROMATOGRAPHY:

1. The extent of concentration (or dilution) of the eluate is dependent on the PCB concentration in the sample being analyzed and the sensitivity and linear range of the EC detector being used. Further concentration will be required for detection with an electrolytic conductivity detector.

2. All samples should be chromatographed on at least two different GLC columns with EC detection to enhance the qualitative aspects of the determination.

NOTE: Further confirmation can be obtained by pooling the 6% Florisil eluates and performing GLC with mass spectroscopy. Pooled samples can also be confirmed by electrolytic conductivity (Cl mode) detection for the presence of PCBs as well as chlorinated pesticides.

3. Primary identification and quantification of PCBs is based on the calculation of all peaks and comparison to an Aroclor 1254 standard. If there is evidence that erroneous data may result from the interference of extraneous compounds, use for quantification only those peaks in the sample chromatogram that are free of interferences. Compare these to the corresponding peaks in the standard chromatogram of Aroclor 1254 (see Section XII and Miscellaneous Note 2).

NOTE: It is likely that standard Aroclor 1254 will yield a chromatogram most closely resembling the array of peaks observed in actual samples. If the sample matches the chromatogram of another Aroclor standard more closely, this compound should be used for quantitative comparisons. See Section 9,E for typical chromatograms of different PCBs.

4. Operating parameters for electron capture GLC are:

Temperatures	-	injector	220-225°C
		columns	200°C
		detector	250-300°C (^{63}Ni) or 205-210°C (^{3}H)
Carrier gas	-	highly purified nitrogen	
Flow rates	-	60-80 ml per minute for OV-17/OV-210 100-120 ml per minute for SE-30/OV-210 (at 40 psig)	

For the electrolytic conductivity detector the temperature parameters are:

injector	245°C
columns	200°C
furnace	820°C
block	230°C

XI. SENSITIVITY AND RECOVERY RESULTS:

The GLC sensitivity limit for PCBs is 20 ppb theoretical and 50 ppb practical based on the whole milk sample weight.

The 50 ppb sensitivity limit was determined in the following manner: Based on the sensitivity of the instrument involved in the analyses and the assumption that background interference was absent, the theoretical detection limit of the method was established as 22 ppb. The average background interference exhibited by the reagent blanks was then added to the theoretical detection limit to give a value of 33 ppb. As a final safety margin, the practical detection limit was established as 50 ppb. Any lower values encountered in the actual samples are reported as "trace."

Three quality assurance samples and a blank (control) were prepared using goat's milk and were analyzed by the procedure at the Colorado State University Laboratory and also at a second laboratory. The fortification levels and average recoveries from at least duplicate analyses on a whole milk basis were as follows:

Sample Content	ppb PCBs Reported (and % Recovery)	
	Colorado Laboratory	Laboratory 2
1. Aroclor 1254, 200 ppb	184 (92%)	137 (68%)
2. Aroclor 1254, 150 ppb	121 (81%)	92 (61%)
3. HCB, 50 ppb; heptachlor epoxide, 60 ppb; p,p'-DDT, 100 ppb; p,p'-DDE, 80 ppb; PCBs, none	0	not analyzed
4. Control	2	14

XII. DISCUSSION OF THE METHOD AND RESULTS:

The average recovery of the Colorado lab shown in the above tabulation for the two PCB samples was 87%, compared to 65% for the second laboratory. The higher recovery results were not unexpected because of the greater familiarity of the Colorado group with their own method. The results of the two laboratories on these split samples are considered to be quite comparable and indicate that the procedure is useful for approximating the level of PCBs in human milk. If known, fortified samples are analyzed with each batch of actual samples; the percent recovery of the samples can be corrected for the recovery of the spikes (see Section XIV,4).

The approximation levels of PCBs reported above were referenced to a commercial standard of Aroclor 1254. Due to the many early eluting gas chromatographic peaks in the reagent blanks and the partial carry-over of p,p'-DDE into the PCB fraction, a maximum of only five out of a total of thirteen GLC peaks for the Aroclor 1254 standard obtained for these chromatograms using the specified packed columns, could be used for the quantifications. In many cases, only the last three eluting GLC peaks could be used in these fingerprinting methods.

The GLC traces generated with the electron capture detector and the two specified GLC columns for the milk samples prepared by the macro Colorado method (and the micro procedure in Section 9,B,(2)) resembled in appearance only the commercial Aroclor 1254 standard. On the contrary, as confirmed by the mass spectrometric analyses, the human milk samples contained a higher percentage of the hexachlorobiphenyl isomers than did the standard Aroclor 1254. The fingerprint chromatogram of each extract resembled the reference standard of Aroclor 1254, but the sample extracts did not contain the equivalent isomeric PCBs. The present inadequacies of the "State of the art" methods using packed GLC columns are the inability to separate and to reference the individual isomers present in the sample extract.

As noted earlier, this method can provide only a semi-quantitative approximation of PCB amounts. Mathematical conversions of such approximation levels of PCBs on a whole milk basis to those on a total fat basis yield numbers with little analytical significance. Extreme care must be applied in considering such approximation levels as indicators of the absolute identity and quantity of PCBs present in human milk.

XIII. MISCELLANEOUS NOTES:

1. The percentage of lipids is calculated as follows: The beaker should be weighed before adding the 20 ml aliquot of sample and again after the hexane has evaporated, leaving the lipid in the beaker. The difference in weight is the weight of the lipid. The weight of the lipid is then divided by the weight of sample in the 20 ml portion to give percent lipid. For a 7.0 g sample, the 20 ml portion would contain 1.4 g sample, and the remaining 5.6 g would be used for PCB analysis.

Example:	Lipid + Beaker:	27.3937 g
	Dry Beaker:	27.3652 g
	Lipid:	0.0285 g

Total milk sample = 7.00 g

$$\text{Sample in 20 ml} = 7.00 \times \frac{20.0}{100} = 1.40 \text{ g}$$

$$\left(\frac{0.0285 \text{ g}}{1.40 \text{ g}}\right) \times 100 = 2.04\% \text{ lipid.}$$

2. An example of the calculation of ppb PCBs on a lipid basis is as follows:

   Original milk sample = 7.00 g
   Milk sample carried through PCB analysis = $7.00 \times \frac{80.0}{100} = 5.60g$
   Final sample volume = 5.00 ml
   Volume injected for GLC - 5.00 μl
   Whole milk sample injected = $5.60 \times \frac{.00500}{5.00} = 5.60$ mg

   Sample peak height(s) (mm) x pg/mm from standard = pg in sample peak(s)

   $$\frac{\text{pg in sample peak(s)}}{\text{5.60 mg sample}} = \text{ppb PCB on whole milk basis}$$

   $$\text{ppb PCB on whole milk basis} \times \frac{100}{2.04} = \text{ppb PCB on lipid basis}$$

   It should be understood that this conversion increases the numerical value but not the analytical significance of the results. An analytically insignificant approximation level of 30-80 ppb of PCBs on a whole milk basis becomes 50 times as great or 1.5 to 4.0 ppm (assuming 2% lipid as above) on a total fat basis. Values at this or lower levels have no significant analytical meaning.

XIV. ANALYTICAL QUALITY CONTROL:

1. If the procedure is being used in a monitoring program, the thoroughness of the personnel collecting the samples in obtaining pertinent data from the donors should be checked periodically. If necessary, further training should be provided.

2. Likely sources of contamination leading to a high reagent blank are the filter paper, glass wool, and sodium sulfate required by the method. These should be thoroughly precleaned with pesticide grade solvents as directed under Subsections IV and V.

3. Each sample analyzed required a total volume of ca 2000 ml of solvent. Care must be utilized in concentration of such a large volume to the final 1-5 ml volume for analysis.

4. A suggested in-house QC program involves running one blank and one fortified sample for every set of 10 human milk samples.

   a. The reagent blank is carried through the entire procedure without addition of milk. Any background interferences displayed by the blank are subtracted from the levels observed in the set of human milk samples.

b. The spiked sample was prepared from 7.0 g aliquots of goat's milk that had been stored in a frozen state at -10°C. Prior to analysis, the aliquot was thawed in a 40°C water bath and spiked with 1.0 ml of an acetone solution containing 200 ng per ml of Aroclor 1254. On a whole milk basis, this represented a PCB concentration of $\frac{200 \text{ ng}}{7.00 \text{ g}} = 28.6$ ppb.

c. The spiked sample is taken through the whole procedure.

d. Typical average recovery from the spiked goat's milk has been 77%, and the average amount of background PCBs has been 78 ng, which is equivalent to 11.0 ppb for a 7.00 g milk sample.

e. Recoveries of the set of actual samples can be corrected for the 77% average recovery from the spiked milk by multiplying each individual recovery by $\frac{100}{77.0}$.

f. At irregular intervals, a goat's milk control sample is analyzed in addition to the reagent blank and spike. Any detectable levels of PCBs would be subtracted from the spiked sample before calculating its recovery level.

5. If an outside, independent source of spiked PCB reference material (SPRM) is available to the laboratory using the procedure, these SPRMs should be used as blind QC checks on analytical performance.

6. Before handling actual samples, laboratory personnel should be guided through the method at least four times by an experienced worker. This should involve analyzing a duplicate of a sample that had already been analyzed by the experienced worker. If the results are acceptable at this point, the individual is allowed to do an additional set of four spiked samples in duplicate without the aid of the experienced worker. After demonstrating adequate results, the individual should be proficient enough to handle actual samples.

DETERMINATION OF PCBs IN HUMAN MILK

MICRO METHODS

I. INTRODUCTION:

This section describes alternative methods for the analysis of PCBs in human milk developed by the Analytical Chemistry Branch of the EPA Environmental Toxicology Division, Health Effects Research Laboratory, Office of Research and Development, at Research Triangle Park, NC, to complement and confirm the macro procedure in Section 9,B,(1). The procedure utilizes chromatography on a micro silicic acid column to further resolve PCBs. As with the method in Section 9,C,(1) these methods are not capable of accurately identifying and quantifying absolute levels of PCBs, but provide semi-quantitative results.

II. PRINCIPLE:

A 0.5 g milk sample is extracted with acetonitrile, residues are partitioned into hexane, and the hexane is concentrated and eluted through a micro Florisil fractionation/cleanup column. The eluate fraction containing PCBs is concentrated and eluted through a micro silicic acid column for further separation of PCBs and chlorinated pesticides. The column eluate containing PCBs is concentrated and analyzed by GLC with electron capture detection. A perchlorination method can be used to confirm the presence and amounts of PCBs.

III. EQUIPMENT AND REAGENTS:

Since this procedure is a combination of methods described in Sections 5,A,(2),(a) and 9,B,(1), readers should refer to the respective subsections on EQUIPMENT and REAGENTS in these Sections. Additional items only will be listed here.

1. a. Centrifuge tube, 40 ml, Corning 8122.

   b. Caps, molded screw, with Teflon liners, size 24-410 (Corning 9999).

   NOTE: Do not use caps which come with Corning 8122 tubes. The rubber liners may contain contaminants.

2. Centrifuge tube, 15 ml.

IV. SAMPLE EXTRACTION:

1. Extract a 500 mg sample of milk with three 2.5 ml portions of acetonitrile in a tissue grinder, and centrifuge at 2000 rpm after each extraction in the grinder.

   NOTES:

   (1) A reagent blank and a fortified sample are started at this point and run through the complete procedure.

   (2) Consult Notes 1 and 2 in Section 5,A,(2),(a),VII.

2. Combine the supernatants in a 40 ml screw cap centrifuge tube.

3. Add 25 ml of 2% aqueous sodium sulfate solution to the combined supernatants and mix on a Vortex mixer.

4. Extract the aqueous acetonitrile mixture in turn with one 5 ml and two 2 ml portions of hexane.

5. Transfer each extract with a disposable pipet and combine in a 15 ml centrifuge tube.

6. Concentrate the hexane solution to a volume of 300-500 µl under a gentle stream of nitrogen.

V. FLORISIL FRACTIONATION:

1. Prepare, prewash, and activate a micro Mills Florisil column according to Section 5,A,(2),(a),III.

2. Remove the column from the oven and allow to cool to room temperature.

3. Prewet the column with 10 ml of hexane and discard the eluate.

   NOTE: From this point on, the solvent level should never be allowed to drop below the top of the bed or channeling may occur and degrade the resolution.

4. Transfer the sample onto the column with a disposable pipet and begin collection of the eluate in a 25 ml concentrator tube.

5. Rinse the sample tube with two 0.5 ml portions of hexane, added with a disposable pipet, and transfer each rinse to the column with the same disposable pipet.

6. Elute the column with an additional 10.5 ml of hexane followed by 12 ml of methanol-hexane (1:99 v/v). All 24 ml are collected in the same concentrator tube and represent Fraction I.

   NOTE: Fraction I contains PCBs and those pesticides listed in this fraction in Table 1, Section 5,A,(2),(a).

7. Elute the column with an additional 12 ml of methanol-hexane (1:99 v/v), collecting the eluate in a second concentrator tube.

   NOTE: This fraction contains heptachlor epoxide and may be checked for any overlap of PCBs. See Table 1, Section 5,A,(2),(a) for the elution pattern of other pesticides from the micro Florisil column.

8. Concentrate Fraction I to 300-500 μl under a gentle stream of nitrogen.

VI. SILICIC ACID FRACTIONATION:

1. Prepare 3% deactivated silicic acid as described in Section 9,B,(1),IX, and prepare a column as follows:
   a. Place one gram in a small beaker and add enough hexane to form a slurry.
   b. Add a small glass wool plug and 10 mm of anhydrous sodium sulfate into a prewashed Chromaflex column, and then pour in the slurry.

      NOTE: Do not allow the solvent level to go below the top of the column bed.

   c. Add another 15 mm of anhydrous sodium sulfate after the silicid acid has settled.
2. Rinse the column with 10 ml of petroleum ether, discarding the eluate.
3. As soon as the last of the rinse reaches the top of the column, transfer the sample onto the column with a disposable pipet and start collecting the effluent in a 15 ml centrifuge tube.
4. Rinse the sample tube with two 0.5 ml portions of petroleum ether, and add the rinses to the column using the same disposable pipet.
5. Collect a total of 4 ml of petroleum ether for Fraction I.

6. Change the collection tube and continue elution until a total of 10 ml of petroleum ether is collected (Fraction II).

   NOTE: Fraction I will contain hexachlorobenzene while Fraction II will contain PCBs and some DDE.

7. Concentrate Fraction II under a gentle stream of nitrogen to a suitable volume for EC GLC.

VII. GAS CHROMATOGRAPHY:

Proceed with electron capture gas chromatography following the general guidelines set forth in Section 4,A,(4) and the specific parameters and procedures in Section 9,B,(1),X. The volumes of sample and standards injected and degree of eluate concentration must reflect the smaller sample size originally taken for the micro method compared to the macro method. Calculations of results are made in a manner similar to that explained in Section 9,B,(1), Miscellaneous Notes 1 and 2. If results are to be reported on a total lipid basis, a separate milk sample is taken for the determination of percentage lipids.

VIII. RESULTS AND DISCUSSION:

Results obtained by the Research Triangle Park Analytical Chemistry Branch with the micro silicic acid procedure on the same fortified goat's milk samples cited in Section 9,B,(1),XI were 162 ppb for the 200 ppb sample (81% recovery) and 139 ppb for the 150 ppb sample (93% recovery). The control yielded 31 ppb PCBs (all results are averages of at least two analyses). In general, the micro method yielded somewhat higher recovery values than did the Colorado macro method for a series of samples analyzed for both. For 10 samples, the macro method gave an average of 66 ppm of PCBs (18-184 ppb range) and the micro method 103 ppb (46-243 ppb range). All results were higher by the latter method except for one sample, which gave the same results by both methods. These results were substantiated by high resolution mass spectroscopic analysis of samples cleaned up by both the macro and micro methods.

Because of the lesser quantities of reagents and smaller glassware required, the reagent blank problem was in general not as great with the micro method. The time required for preparing a set of samples with the micro method was considerably less than for the macro method. However, confirmational analyses of the micro method extracts were limited owing to the small sample size (see Miscellaneous Note 1). The micro method seemed to respond, although with wide degrees of variations, to lower levels than did the macro method.

It must be emphasized again that the micro and macro methods are both semi-quantitative, yielding approximate PCB values. Extreme care must be applied in considering such approximation levels as indicators of the absolute identity and quantity of PCBs in human milk.

IX. MISCELLANEOUS NOTES:

1. Chemical derivatization by perchlorination to yield decachlorobiphenyl (DCB) followed by EC GLC has been used to confirm PCBs in selected samples of human milk which had been cleaned up by either the macro or micro methods. The procedure, as reported by Crist, H. L. and Moseman, R. F., in J. Assoc. Off. Analy. Chem., 60, 1277 (1977), follows:

   a. Transfer a cleaned-up sample equivalent to as much as 500 mg of milk to a 1.5 ml glass tube fitted with a Teflon screw cap (Mini-Actor, Applied Science Laboratories). Standards and blanks should be run with each set of samples.

   b. Evaporate the solvent to 0.02-0.03 ml under a gentle nitrogen stream. Add ca 0.25 ml of chloroform and evaporate again to 0.02-0.03 ml. Repeat chloroform addition and evaporation twice more to ensure complete removal of hydrocarbon solvent.

      NOTES:

      1. Hydrocarbon solvents react with $SbCl_5$ to form a carbonaceous mass.

      2. Avoid complete dryness of the sample to prevent loss of PCBs with a lower degree of chlorination.

   c. Add by pipet exactly 0.2 ml of antimony pentachloride (J. T. Baker).

   d. Tightly cap the tube and place in a sand bath at 160-170°C for 16 hours.

   e. Remove the tube, allow to cool, and carefully remove the cap. Add ca 0.5 ml of 6 M hydrochloric acid to the tube to deactivate excess reagent.

   f. Transfer the mixture to a 15 ml centrifuge tube with a disposable Pasteur pipet.

g. Rinse the reaction tube with an additional 0.5 ml of the HCl and three 0.5 ml portions of hexane, transferring all rinses to the centrifuge tube with the same disposable pipet. Rinse the reaction tube cap with several drops of acid and hexane in the same manner to complete transfer of the sample.

h. Extract the DCB from the acid phase into the hexane by mixing for 30 seconds on a Vortex mixer.

i. After phase separation, transfer the hexane to a clean 15 ml centrifuge tube using a new disposable pipet. Repeat the extraction twice with 1.0 ml portions of hexane.

j. Wash the combined hexane extracts with 1.0 ml of distilled water for 30 seconds, followed by 1.0 ml of 10% aqueous sodium bicarbonate solution. Discard both aqueous wash phases.

k. Reduce the hexane volume to ca 0.25 ml under a gentle nitrogen stream.

l. Remove a prewashed and activated micro Florisil column from the oven (Section 5,A,(2),(a),III) and let cool.

m. Prewet the column with 10 ml of hexane. After draining the wash solvent to the top of the bed, transfer the sample quantitatively to the column with a disposable pipet, rinsing with two 1 ml portions of hexane and collecting the eluate in a 15 ml centrifuge tube.

n. Elute DCB from the column with a total of 7 ml of hexane.

NOTE: Elution patterns for DCB should be verified by the analyst to take into account variation in Florisil activity.

o. Reduce the eluate to an appropriate volume.

p. Inject a sample into an electron capture gas chromatograph fitted with a 1 m x 4 mm i.d. column of 5% OV-210 at 200°C and a carrier gas flow rate of 60 ml per minute. DCB has a retention time of ca 8 minutes under these conditions.

q. Determine the amount of DCB in the sample. Convert to the desired Aroclor by multiplying DCB found by the quotient obtained from dividing the average molecular weight of the Aroclor by 499, the molecular weight of DCB.

NOTE: Since all Aroclors have an average molecular weight less than 499, levels of PCBs expressed as a particular Aroclor will always be less than than calculated for DCB.

The perchlorination method gave values of 145 ppb for a 200 ppb fortified milk standard and 102 ppb for a 150 ppb standard cleaned up by the macro method (after correction for a 37 ppb reagent blank), based on the amount of Aroclor 1254 that would produce an equivalent amount of DCB. This indicates that the method is capable of providing a further semi-quantitative approximation of PCBs in human milk samples. Results are not expected to be totally consistent between the EC GLC fingerprinting method using Aroclor 1254 as reference standard and DCB method because only later eluting peaks are useful for quantitative approximation in the former (because early peaks of less chlorinated isomers would be overlapped by impurities and would not resemble the standard chromatogram), whereas all isomer peaks will contribute to the production of DCB. The greatest inadequacy of the perchlorination technique is the inability to determine the isomeric identity and distribution in an unknown and the total conversion efficiency of each of the different isomers to DCB.

The following table shows comparisons between results obtained for human milk samples by the perchlorination method and a miniaturized silicic acid cleanup procedure preceding EC GLC. The results indicate reasonably good agreement at the low concentrations present.

X. ANALYTICAL QUALITY CONTROL:

General aspects of quality control for the micro method are similar to those presented for the macro method in Section 9,B,(1),XIV, with details modified to reflect the smaller sample size taken through the procedure. A typical SPRM to be analyzed along with actual samples would contain 15 ng/500 mg = 30 ppb.

POLYCHLORINATED BIPHENYLS IN HUMAN MILK EXTRACTS (500 mg)

	PCBs found, ppm (as Aroclor 1254)[a]	
Sample	Perchlor	Micro
1[b]	0.12	0.15
2[c]	0.14	0.17
3	0.05	0.04
4	0.06	0.07
5	0.08	0.09
6	0.06	0.06
7	0.06	0.06
8	0.04	0.05
9	0.14	0.19
10	0.07	0.10

[a] Reported on whole milk basis.

[b] Goat milk fortified with 0.15 ppm Aroclor 1254.

[c] Goat milk fortified with 0.20 ppm Aroclor 1254.

Samples 3-10 were human milk samples.

Figures 1 and 2 illustrate a human milk sample before and after perchlorination. Perchlorination combined with the micro extraction and cleanup method is particularly well suited for confirmatory purposes when the amount of sample is limited. The cleanup achieved allows perchlorination of the sample without excessive lipid interference with the reaction.

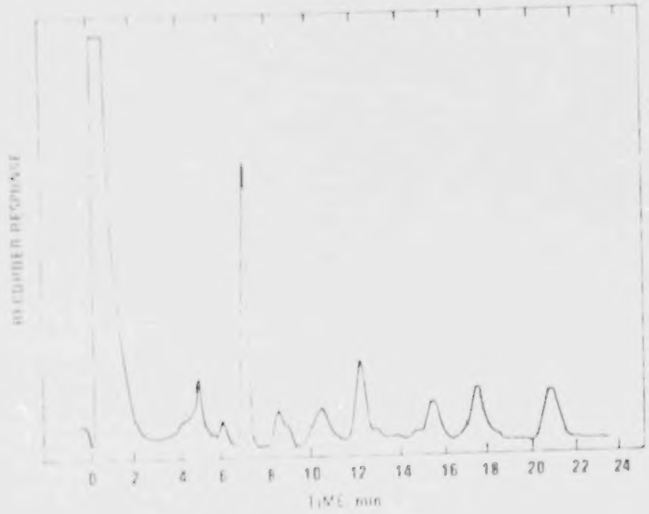

Fig. 1. Chromatogram of human milk extract; 5 µl/1.0 ml injected: 4% SE-30/6% OV-210 column; oven temperature 205°C; carrier gas flow 60 ml/minute.

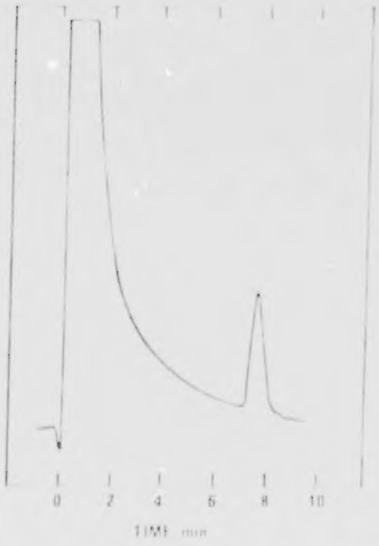

Fig. 2. Chromatogram of perchlorinated human milk extract (0.14 ppm as Aroclor 1254); 5 µl/8.0 ml injected; OV-210 column; oven temperature 205°C; carrier gas flow 60 ml/minute.

X. ANALYTICAL QUALITY CONTROL:

General aspects of quality control for the micro method are similar to those presented for the macro method in Section 9,B,(1), XIV, with details modified to reflect the smaller sample size taken through the procedure. A typical SPRM to be analyzed along with actual samples would contain 15 ng/500 mg = 30 ppb.

SEPARATION OF SOME POLYCHLORINATED BIPHENYLS FROM CERTAIN ORGANOCHLORINE PESTICIDES

I. INTRODUCTION:

Polychlorinated biphenyls (PCB) are a group of chemicals with industrial applications. They are stable (resistant to alkali and acid) and persistent; their residues have been found in wild life. Most Aroclors actually consist of many different chlorobiphenyls, although some, partially or totally, consist of members of another group of compounds, chloroterphenyls.

The various components of PCB residues are partially or completely recovered through multiresidue methodology for organochlorine pesticides; they are eluted from the Florisil column by the 6% ethyl ether/petr ether eluant. PCB residues exhibit complex gas chromatographic patterns because of the various components represented. These peaks appear in and beyond the retention time region of the organochlorine pesticides. If present in high enough concentration, relative to pesticides present, PCB can interfere with the determination of some organochlorine pesticides. Likewise, the presence of certain organochlorine pesticides can interfere with the determination of PCB.

NOTE: The polychlorinated terphenyls (PCT) are also recovered through the multiresidue methodology used for the analysis of organochlorine pesticides and PCB. However, the PCT elute from the GLC column much more slowly than either the pesticides or PCB and so do not interfere with the determination. In order to analyze for the PCT, it is necessary to use a GLC column and operating parameters which permit much more rapid elution and greater sensitivity for the chloroterphenyl components.

A number of procedures have been proposed for dealing with the various pesticide PCB combinations encountered. These include the silic acid column chromatographic separation technique presented in detail here and several other published approaches, some of which are noted in Subsection VII. The residue analyst must make judicious use of the available techniques in order to obtain accurate results. The proper course of action in the determination of residues of PCB and pesticides found together depends on the suspected identity of each and on the estimated amounts of each. Some combinations will permit quantitation of both pesticides and PCB without their separation from one another on the silicic acid column. Other combinations of PCB and pesticides must be separated before quantitation; still other combinations cannot be separated by silicic acid, yet cannot be determined in the presence of one another. The relative amounts of residues of pesticides and PCB may also influence the decision on whether or not to perform silicic acid separation prior to quantitation. Even when the residues will not be completely separated by this technique, its use may be the best means of achieving quantitative estimation of the residues when one chemical is present in much larger amounts than the other.

REFERENCES:

1. Armour, J., and Burke, J., JAOAC 53, 761-767 (1970).
2. Masumoto, H. T., JAOAC, in press.
3. Pesticide Analytical Manual, Vol. 1, Section 251, U. S. Food & Drug Admin.

II. PRINCIPLES:

The silicic acid column chromatographic procedure given here permits separation of DDT and its analogs from some of the PCB, including those with which they interfere. The silicic acid is standardized before use by addition of enough water to effect the best possible separation between p,p'-DDE and Aroclor 1254. The interfering PCB are eluted with petroleum ether from a column of the standardized silicic acid. The DDT compounds, most other organochlorine pesticides, and some other PCB are then eluted from the column with a mixture of hexane, methylene chloride, and acetonitrile. See Table I for list of chemicals eluting in each of the two eluates.

The method is applicable to the 6% Florisil eluate [Section 5,A,(1)] obtained in the analysis of fatty tissues or to extracts cleaned up by other methods for gas chromatography. Extracts in polar solvents must be transferred to nonpolar solvents prior to separation.

III. APPARATUS:

1. Chromatographic column 400 x 22 mm i.d., with 24/40 ₮ outer joint with coarse fritted plate and Teflon stopcock, Kontes No. K-420550, C-4, or the equivalent.

2. Grad. Cylinder, 250 ml.

3. Kuderna-Danish Assembly as follows:

Evaporative concentrator flask - Kontes Catalog No. K-570000, 500-ml capacity, lower joint 19/22 ₮, upper joint 24/40 ₮;
Snyder column - 3 ball, lower joint 19/22 ₮, upper joint 24/40 ₮;

Tube - 10 or 15 ml capacity, 19/22 $ upper joint.

4. Air pressure regulator - for pressure reduction to deliver ca 1 lb. psig; air must be clean and dry.

5. Separatory funnel - Used for column eluant reservoir, 250 ml, with Teflon stopcock, 24/40 $ joint at top, Kontes No. K-633030, or the equivalent.

6. Hot water bath adjustable to temp. of 90-100°C.

IV. REAGENTS:

1. Petroleum ether, acetonitrile, hexane, and methylene chloride, all pesticide quality.

2. Celite 545, Johns-Manville.

3. Silicic acid, Mallinckrodt, 100 mesh powder; "specially prepared for chromatographic analysis by the method of Ramsey and Patterson," Analyt. Reagent No. 2847.

V. PREPARATION OF SPECIAL REAGENTS:

1. Celite 545 - must be dry and free of electron capturing substances. If electron capturing substances are extracted by petroleum ether, treat as follows: Slurry Celite with 1 + 1 hydrochloric acid - H_2O while heating on steambath; wash with H_2O until neutral; wash successively with several portions each of methanol and acetone (to remove H_2O); then ethyl acetate and petr ether. Remove solvents by suction and air drying. Hold a 1- to 2-inch layer of Celite in 130°C oven for at least seven hours to remove water and other volatile substances. After washing treatment and/or drying, store Celite in closed glass container.

2. Eluant for Pesticides - 1% acetonitrile, 19% hexane, 80% methylene chloride (v/v/v). Pipette 10 ml acetonitrile into 1-liter volumetric flask, add 190 ml hexane, and fill to volume with methylene chloride.

3. Silicic acid - Place silicic acid to depth of about 1 inch in open beaker and heat for a minimum of 7 hours, but preferably up to 24 hours, in 130°C oven to remove water. After heating, place beaker in desiccator and allow to cool to room temperature. Quickly weigh silicic acid into glass-stoppered bottle and add 3% H_2O by pipette (97 g silicic acid + 3 ml H_2O = 3% H_2O). Stopper bottle tightly and seal with tape to insure that container

is air tight. Shake well until all H_2O is absorbed; make sure that no lumps remain. Place sealed container in desiccator and allow to equilibrate for 15 hours.

To determine the separation achieved with the treated silicic acid, prepare a column as described in VI, below, and add to it a standard solution containing 40 μg Aroclor 1254 and 3 μg p,p'-DDE in hexane. Elute as described and determine recoveries in each eluate. Inadequate separation of PCB from p,p'-DDE will require that further testing be done with other heated batches of silicic acid, treated with different amounts of H_2O as needed to achieve the desired separation. Increments of 0.25% or 0.5% more or less H_2O are recommended for the testing. More H_2O is required when the initial test results show PCB eluting in the polar solvent with the p,p'-DDE; less H_2O when p,p'-DDE elutes in the petr ether fraction.

This testing and standardization is required for each new lot of silicic acid obtained from the manufacturer.

Once a batch of standardized silicic acid is prepared, it should be stored in a desiccator between uses. Desired activity remains for about 5 days.

VI. SEPARATION OF PCBs FROM ORGANOCHLORINE PESTICIDES:

1. Weigh 5 g Celite, then 20 g activated silicic acid and combine in 250 ml beaker. Immediately slurry with 80 ml petr ether, mixing well.

2. Pour slurry into a chromatographic column with coarse frit, keeping stopcock open. Complete transfer of silicic acid - Celite mixture by rinsing beaker with small portions of petr ether.

   NOTE: Apply air pressure to top of column as much as necessary to force enough petr ether from column to allow space for all silicic acid - Celite.

3. Stir material in column with long glass rod to remove air bubbles, applying air pressure to settle adsorbent and to force petr ether through column. Continue application of air pressure until petr ether level is ca 3 mm above surface of gel.

   NOTE: Do not allow column to go dry or to crack at any time during the procedure. Close stopcock when air pressure is not being applied. At this point, column of adsorbent should be firm and should not lose its shape if tipped.

4. Place 250 ml grad. cylinder under column for collection of eluate and take a suitable aliquot of 6% Florisil extract for addition to the column.

   NOTE: Large amounts of PCB and pesticides placed on column may result in incomplete separation. Choose aliquot to contain amounts of PCB and pesticides required for determination. The weight of sample equivalent placed on the column may also affect separation by causing p,p'-DDE to appear in the petr ether eluate. Should this occur, an amount of extract equivalent to a smaller weight of sample should be used. In analysis of samples by this procedure, it is suggested that no more than 0.3-0.4 g fat equivalent be placed on the silicic acid column.

5. Add aliquot carefully to column being careful not to disturb top of adsorbent.

6. Apply slight air pressure until solvent level is ca 3 mm above adsorbent and then complete transfer of sample extract to column using small portions of petr ether and again applying slight air pressure until solvent level again reaches the 3 mm point above adsorbent.

7. Position a 250 ml sep. funnel containing 250 ml of petr ether on top of column. Open funnel stopcock and slowly apply air pressure to reservoir until an elution rate of ca 5 ml/min. is established. Continue elution until eluate volume in the graduate is exactly 250 ml.

8. Quantitatively transfer eluate to a 500 ml Kuderna-Danish evaporator fitted with a 5 ml evap. concentrator tube. Rinse graduate with small portions of pet. ether.

9. Place a second 500 ml K-D flask assembly under the column for collection of any remaining petr ether eluant and second eluate described in Step 10.

10. Apply air pressure until petr ether eluant level is ca 3 mm above adsorbent and add 200 ml of CH_3CN-hexane-CH_2Cl_2 (1:19:80) eluant to upper reservoir. Open stopcock and slowly reapply air pressure, continuing elution until all of eluant passes through column into the C-D concentrator.

11. Place Snyder columns on both K-D assemblies, place in a hot water bath and reduce eluate volumes to 5 ml in preparation for exploratory GLC analyses.

NOTE: The first (petr ether) eluate should contain the PCB's and the combined solvent eluate should contain the chlorinated pesticides.

VII. GAS CHROMATOGRAPHY AND INTERPRETATION:

Determine quantity of PCB in the sample by electron capture GLC or by halogen specific microcoulometric or electrolytic conductivity GLC. Compare the total area of response for the residue to the total area of response for a known weight of the Aroclor(s) reference with most similar GLC pattern(s). The pattern of GLC peaks for a sample containing PCB is often not exactly like that from any of the Aroclor standards. This is probably due to a combination of circumstances, e.g., weathering and/or metabolism of the residue; and perhaps slight variation in the recovery of the different PCB components through the methodology. Sometimes the GLC curve clearly indicates the presence of components of more than one Aroclor. In this case, quantitate the PCB residues separately if possible, using the appropriate Aroclor references for the respective portions of the GLC curve. Choosing the appropriate Aroclor reference(s) against which to measure a residue requires good judgment on the part of the analyst.

GLC with halogen specific microcoulometric or electrolytic conductivity detection is often preferable as means of quantitation. This type of detection system also provides confirmatory evidence for the identification of the residue as PCB.

TABLE 1. PESTICIDES AND OTHER CHEMICALS RECOVERED THROUGH SILICIC ACID COLUMN CHROMATOGRAPHIC SEPARATION OF SOME POLYCHLORINATED BIPHENYLS (PCB) FROM CERTAIN ORGANOCHLORINE PESTICIDES.[a]

Petroleum Ether Eluate	Acetonitrile, Methylene Chloride, Hexane Eluate
Aldrin	Aroclor 1221[b]
Aroclor 1221[b]	Aroclor 1242[b]
Aroclor 1242[b]	Aroclor 1248[b]
Aroclor 1248[b]	Aroclor 5442[d]
Aroclor 1254	Aroclor 5460[c,d]
Aroclor 1260	BHC (all isomers)
Aroclor 1262	chlordane (technical)
Aroclor 4465	*p*,*p*'-DDE
Aroclor 5460[c,d]	*o*,*p*'-DDT
hexachlorbenzene	*p*,*p*'-DDT
mirex[e]	dieldrin
octachloro-dibenzo-p-dioxin	endrin
polychlorinated naphthalenes[f]	heptachlor
2,3,7,8-tetrachloro-dibenzo-p-dioxins	heptachlor epoxide
	lindane
	Perthane[g]
	p,*p*'-TDE
	toxaphene

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[a] Method tested only with chemicals listed.

[b] Divides between the two eluates. The earliest (GLC) eluting peaks in any of these Aroclors are the most likely to elute in the polar eluate.

[c] Divides between the two eluates.

[d] Aroclors 5442 and 5460 are composed of polychlorinated terphenyls and must be chromatographed on a GLC column that permits rapid elution; e.g., 1% OV-101 on 100/120 Gas Chrom Q at 240°C, 120 ml N_2/min. (Wieneke, W., private communication, Jan. 1972).

[e] Mirex may be separated from Aroclors 1260 and 1254 by collecting the first 100 ml petr ether separately. This fraction will contain the mires. (Gaul, J., private communication, July 13, 1971).

[f] Method tested with commercial polychlorinated naphthalenes: Halowaxes 1014, 1099 (Armour, J., and Burke, J., JAOAC 54, 175-177 (1971).

[g] Krause, R. T., in press. JAOAC.

SEMI-QUANTITATIVE ESTIMATION OF POLYCHLORINATED BIPHENYLS IN ADIPOSE TISSUE

I. INTRODUCTION:

The incidence of certain of the polychlorinated biphenyls (PCB's) in human adipose tissue has become quite common in very recent years although the compounds have been in use nearly 40 years. It seems probable that improved methods of detection may well account for the prevalence of the current observations.

The prime manufacturer in the United States of these products is the Monsanto Chemical Company. A series of the PCB's are marketed under the trade name of Aroclor. A company bulletin lists many products in which the materials may be used as plasticizers, flame retardants, insulating fluids, or to import some other useful quality. Among these products are natural and synthetic rubber, electrical products, floor tile, printer's ink, coatings for varnishes, waxes, asphalt and many adhesives and resins. The PCB's have also been recommended by Monsanto for mixing with chlorinated insecticides to suppress their vaporization and extend their kill-life.

The Aroclor series of compounds are identified by numbers such as 1242, 1248, 1254, 1260, and so on. The last two digits of the formulation indicate the percentage of chlorine. To date, the two compounds which have predominantly appeared in adipose tissue samples are Aroclor 1254 and 1260. The method presented here essentially is a modification of the method developed by Mulhorn et. al., (1), provides a convenient means of separating these compounds from the common chlorinated pesticides. confirming identification, and approximating the concentration. In addition to Aroclor 1254 and 1260, the method is also applicable to aroclor 1262 and 1268.

Thin layer chromatography provides a sound approach for the semi-quantitation of the stated PCB's as the various compounds of the series have similar Rf values and therefore, produce a single spot.

REFERENCES:

1. Mulhorn, Cromartie, Reichel, and Belisle
Semi-Quantitation of Polychlorinated Biphenyls in Tissue Samples by Thin Layer Chromatography presented at the 84th meeting of the AOAC, October 12-15, 1970 in Washington, D. C. #8

2. Private communication from Monsanto Tentative Procedure for the Determination of Airborn Polychlorinated Biphenyls.

3. Pionke, Chesters, and Armstrong Dual Column and Derivative Techniques for Improved Specificity of Gas-Liquid Chromatographic Identification of Organochlorine Insecticide Residues in Soil Analyst October 1969, 94, pp 900-903.

4. W. W. Sans - Multiple Insecticide Residue Determination Using Column Chromatography, Chemical Conversion, and Gas-Liquid Chromatography J. Ag. Food Chem 15 Jan-Feb 1967, pp 192-198.

II. PRINCIPLES:

Adipose tissue is subjected to extraction by pet. ether, acetonitrile partitioning, and Florisil cleanup. A portion of the resulting 6% ethyl ether/pet. ether eluate, in concentrate form, is treated with KOH to effectuate dehydrochlorination of DDT and DDD to their olefins, thus eliminating the problem of separating these pesticides from the PCB's. Oxidative treatment is then applied to convert any interfering DDE to p,p'-dichlorobenzophenone which has an Rf value different from the PCB's. The PCB's are then determined by thin layer chromatography.

III. APPARATUS:

1. Gas chromatograph fitted with E. C. detector (this equipment is not mandatory for this specific method unless assessment of the pesticides is required).
2. Evap. concentrator tubes, 10 ml, size 1025, Kontes #570050.
3. Evap. concentrator tubes, 25 ml, size 2525, Kontes #570050.
4. Modified micro-Snyder columns, ₮ 19/22, Kontes #569251.
5. Glass beads, 3 mm plain, Fisher #11-312, or the equivalent.
6. Pipets, disposable (Pasteur), 9-inch length.
7. Pipets, spotting, 10 µl, Kontes #763800.
8. All equipment specified in section 5,A,(1) of this manual for the extraction and cleanup of adipose tissue.

9. Equipment specified in Section 12,B of this manual to conduct thin layer chromatography.

10. A bath of white mineral oil and heating device with sufficient control to hold bath at 100°C., ±2°. A beaker resting on a rheostatically controlled electric hot plate may be used.

11. Steam or hot water bath adjustable to 95 to 100°C.

12. Vortex mixer, variable speed.

IV. REAGENTS AND SOLVENTS:

1. Hexane, pesticide quality.

2. Benzene, pesticide quality.

3. Ethanol, absolute.

4. Methanol, absolute.

5. Acetic acid, glacial, reag. grade.

6. Chromium trioxide, cryst., reag. grade.

7. Potassium hydroxide, pellets, reag. grade.

8. Silver nitrate, cryst., reag. grade.

9. Developing solvent 5% benzene in hexane.

10. Aluminum oxide G (Merck).

11. Alcoholic KOH, 2.5% w/v of KOH in ethanol - As this reagent should be prepared fresh each day of use, it is convenient to prepare only a small quantity. One pellet (ca 80 ml) is dissolved in 3 ml of ethanol. Five minutes of vigorous mixing should suffice to complete solution.

12. Oxidizing solution - 1.5 grams of CrO_3 is added to 1 ml of distilled water. Finally add 59 ml of glacial HAC. This solution should be suitable for a month's use.

13. TLC plate coating - Dissolve 1 gram of $AgNO_3$ in 4 ml of distilled water and add 56 ml of methanol. Mix this with 30 grams of Al_2O_3 -G and prepare 8" TLC plates as described in Section 12,B of this manual.

14. Analytical reference standards of the series of Aroclor compounds available from Perrine Repository.

15. a. Stock Standard solution, Aroclor 1260. Weigh 50 mg, dissolve in benzene and dilute to 50 ml. Concentration is 1 mg/ml.

    b. From the stock standard, prepare four working standards of 25,50,100 and 400 ng/μl, using hexane as the diluent.

V. EXTRACTION AND CLEANUP:

An adipose tissue sample of sufficient size to yield 3 grams of pure fat is prepared, extracted, and carried through acetonitrile partitioning and Florisil cleanup as described in Section 5,A,(1) of this manual, altering the latter procedure only by using a 25 ml evap. concentrator tube for the final evaporation. For the purpose of the following procedure, only the concentrate from the 6% ethyl ether/pet. ether eluate is needed as the PCB's are eluted in this fraction. Pipet off an aliquot representing 5% of the extract for such direct GLC analysis as may be required. Use the 95% remaining in the 25 ml evap. concentrator tube for dehydrochlorination.

VI. DEHYDROCHLORINATION:

1. Attach a modified micro Snyder column to the concentrator tube and concentrate the extract to 1 ml or less in a 100°C water or steam bath.

2. Cool and remove micro Snyder column.

3. Remove the volatile solvent under a stream of nitrogen at room temperature and add 2 ml of alcoholic KOH.

4. Reattach the modified micro Snyder column and immerse tube in a 100°C oil bath for 30 minutes.

   NOTE: Do not attempt to use a hot water or steam bath for this purpose.

5. Remove tube from oil bath, allow to cool to room temp. and add 2 ml of dist. water and 5 ml of hexane. Stopper and mix vigorously on a Vortex mixer for 30 seconds.

6. Allow layers to separate and, with a disposable pipet, carefully transfer the hexane layer to a 25 ml evap. concentrator tube.

7. Add 5 ml portions of hexane for two additional extractions as described above in Steps 5 and 6.

8. Adjust the final volume exactly to 19 ml, stopper and mix vigorously on Vortex mixer for one minute.

9. Transfer 1 ml (representing 5% of the original extract) to a second 25 ml evap. concentrator tube, add exactly 10 ml of dist. water, stopper, mix thoroughly on the Vortex, and set aside for direct injection of the hexane layer for GLC assessment.

10. Add a 3 mm glass bead to the first 25 ml evap. concentrator tube containing the remaining 90% of the hexane extract, attach a modified micro Snyder column and boil down to 1 ml or less in a steam or hot water bath.

11. Take tube from bath, allow to cool and remove column. Place the tube under a nitrogen stream and evaporate to dryness at room temperature.

VII. OXIDATION:

1. Add 2 ml of the oxidizing solution to the tube, attach a modified micro Snyder column and immerse tube in the 100°C oil bath for 30 minutes.

2. Remove tube from oil bath, allow to cool, and add 10 ml of dist. water and 3 ml of hexane. Stopper and mix vigorously on Vortex for 30 seconds.

3. Allow layers to separate and carefully transfer the hexane layer to a 10 ml evap. concentrator tube with a disposable pipet fitted with rubber bulb.

4. Add 3 ml portions of hexane for two additional extractions as described above in Steps 2 and 3.

NOTE: If GLC analysis for the dichlorobenzophenone is required, adjust the volume of extract to exactly 9 ml, stopper, mix vigorously on Vortex 30 seconds and transfer 0.5 ml to a 25 ml evap. concentrator tube. Add 9 ml of dist. water, stopper, mix vigorously and hold for direct injection of the hexane extract into the GLC.

5. Add one 3 mm glass bead to the tube, attach a modified micro Snyder column and concentrate the extract to 0.3 ml in a boiling water bath.

6. Remove, allow tube to cool, rinse column joint with ca 2 ml of hexane, stopper and hold on Vortex at medium speed for 30 seconds.

7. Place tube under a nitrogen stream and evaporate just to dryness at room temp. Add exactly 0.1 ml of hexane, stopper and mix on Vortex for 1 minute.

   NOTE: From a 3.0 gm. sample of pure fat, assuming that aliquots were removed for dichlorobenzophenone and GLC before and after dehydrochlorination, the sample weight equiv. in this final 100 μl of extract is 25.5 mg per microliter.

VIII. THIN LAYER CHROMATOGRAPHY:

1. On one 8 in. T.L. plate, spot 10 μl each of the four working standards of Aroclor 1260 and also 10 μl of the concentrated extract from Step 7 under the OXIDATION subsection above.

2. Develop the plate in 200 ml of a solution of 5% benzene in hexane to a previously scored line 150 mm from the spotting line.

3. Remove plate from tank and allow solvent to evaporate.

4. Expose plate in the U. V. box until the sample spot is clearly visible.

5. Remove plate from U. V. box and, by visual comparison of sample spot intensity to the intensities of the various standard spots, estimate the number of nanograms represented by the sample spot.

   NOTE: The operator should be comparing varying degrees of intensity of a gray shading. If the sample spot is black, the indication is an excessive concentration of sample, and quantitative comparisons are not possible. In this case, some quantitative dilution of the sample extract is required to reduce the spot intensity to a level comparable with the standards.

IX. MISCELLANEOUS NOTES:

1. Any p,p'-DDT present in the sample may be measured by GLC quantitation of the p,p'-DDE peak before and after dehydrochlorination. Also, any o,p'-DDT present in the sample may be quantitated by measurement of the o,p'-DDT peak before and after dehydrochlorination.

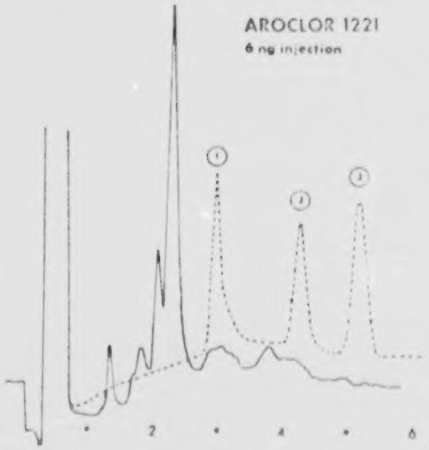
2. While it may be possible to detect and estimate lower levels, an arbitrary limit of ca 1.0 ppm has been tentatively established for this procedure.

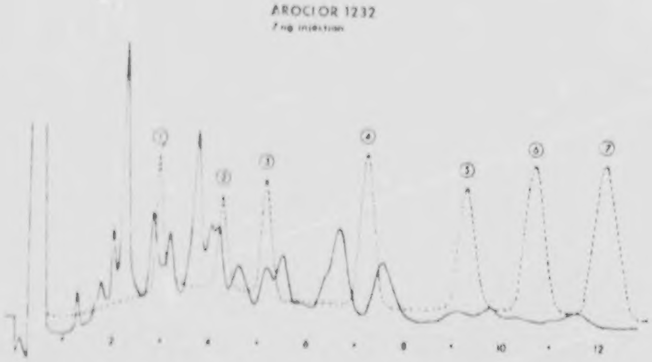
3. Recovery studies have indicated a precision of ±50% for this procedure when using Aroclor 1260 as the reference standard.

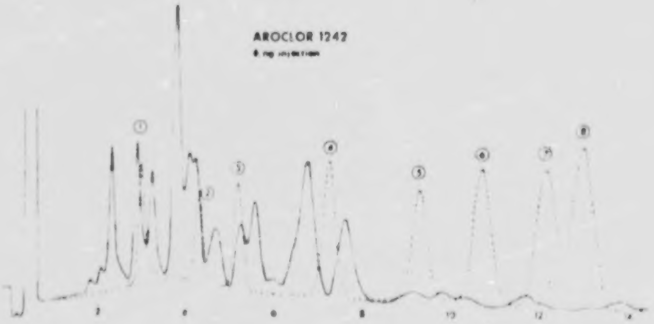
4%SE-30/6%OV-210

Chromatograms of three AROCLORS on column of 4% SE-30 / 6% OV-210. Column temp. 200°C., carrier flow 60 ml/min., ^3H detector, electrom. attenuation on an E-2 10 x 16; dotted line a mixture of chlorinated pesticides, identity and injection concentration given below:

1.	Diazinon	-- 1.5 ng	7.	o,p'-DDT	-- 0.24 ng
2.	Heptachlor	-- 0.03	8.	p,p'-DDD	-- .24
3.	Aldrin	-- .045	9.	p,p'-DDT	-- .30
4.	Hept.Epox.	-- .09	10.	Dilan	-- .75
5.	p,p'-DDE	-- .09	11.	Methoxychlor	.60
6.	Dieldrin	-- .12			



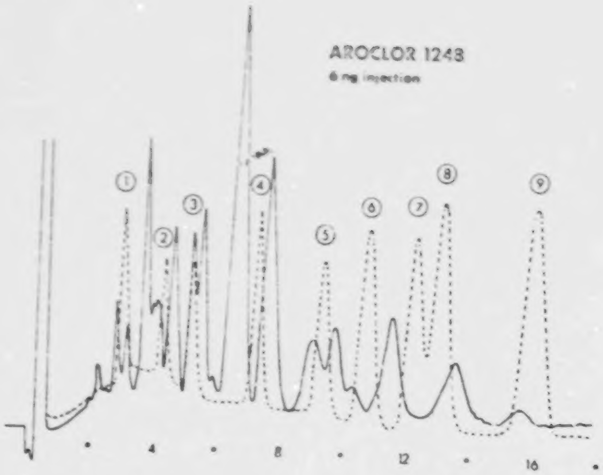


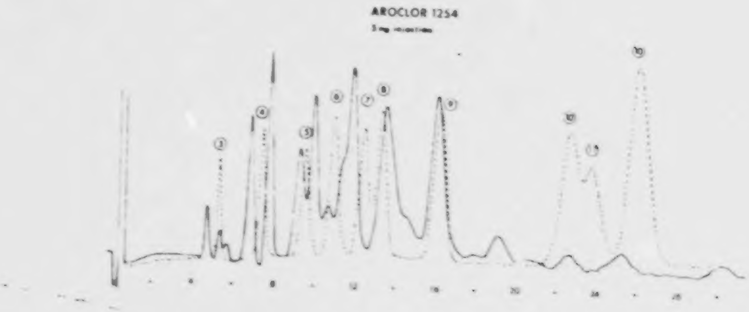


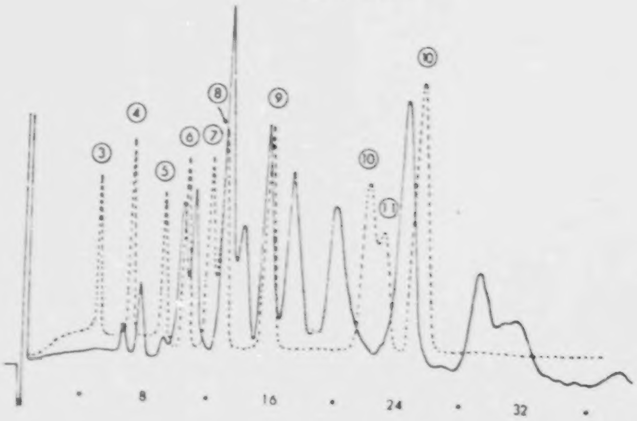
4%SE-30/6%OV-210

Chromatograms of three AROCLORS on column of 4% SE-30 / 6% OV-210. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrom. attenuation on an E-2 10 x 16; dotted line a mixture of chlorinated pesticides, identity and injection concentration given below:

1. Diazinon -- 1.5 ng
2. Heptachlor -- 0.03
3. Aldrin -- .045
4. Hept.Epox. -- .09
5. p,p'-DDE -- .09
6. Dieldrin -- .12
7. o,p'-DDT -- 0.24 ng
8. p,p'-DDD -- .24
9. p,p'-DDT -- .30
10. Dilan -- .75
11. Methoxychlor .60



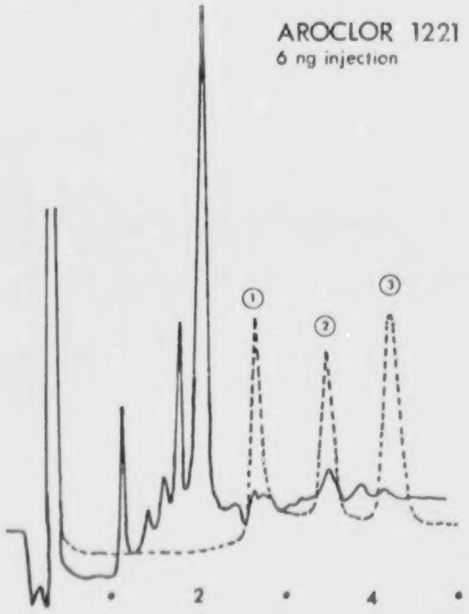


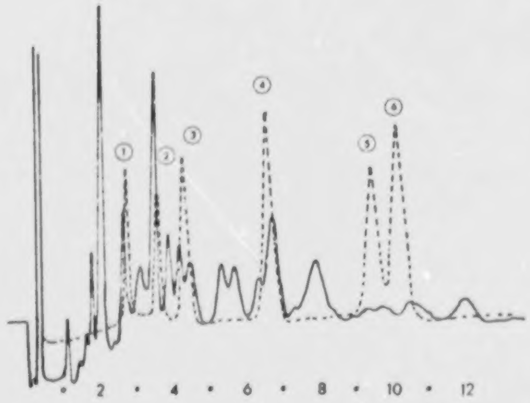


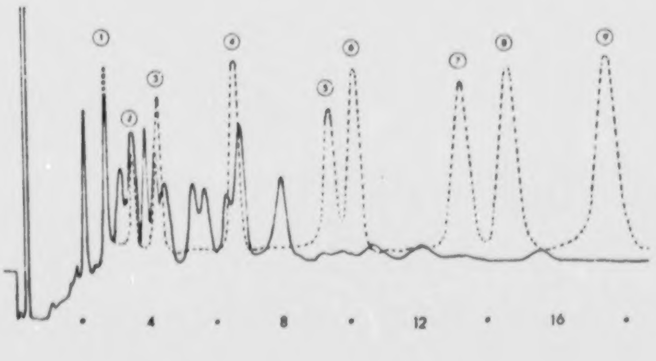
1.5% OV-17/1.95% QF-1

Chromatograms of three AROCLORS on column of 1.5% OV-17 / 1.95% QF-1. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrometer atten. 10 x 16 on an E-2; dotted line a mixture of chlorinated pesticide compounds, identity and injection concentration given below:

1.	Diazinon	-- 1.5 ng	7.	o,p'-DDT	-- 0.24 ng
2.	Heptachlor	-- 0.03	8.	p,p'-DDD	-- .24
3.	Aldrin	-- .045	9.	p,p'-DDT	-- .30
4.	Hept.Epox.	-- .09	10.	Dilan	-- .75
5.	p,p'-DDE	-- .09	11.	Methoxychlor	.60
6.	Dieldrin	-- .12			


AROCLOR 1221
6 ng injection


AROCLOR 1232
7ng injection

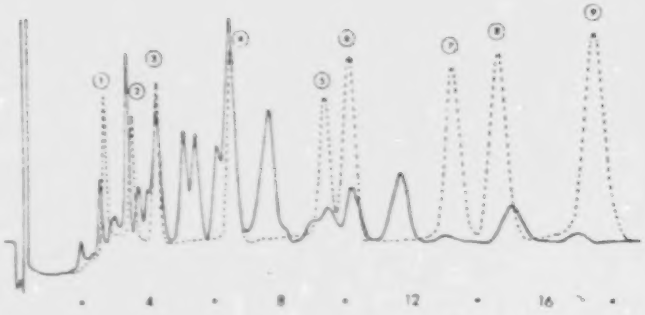

AROCLOR 1242
5 ng injection

1.5% OV-17/1.95% QF-1

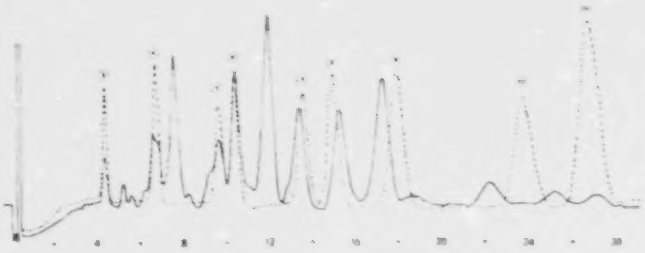
Chromatograms of three AROCLORS on column of 1.5% OV-17 / 1.95% QF-1. Column temp. 200°C., carrier flow 60 ml/min., ^{3}H detector, electrometer atten. 10 x 16 on an E-2; dotted line a mixture of chlorinated pesticide compounds, identity and injection concentration given below:

1. Diazinon -- 1.5 ng
2. Heptachlor -- 0.03
3. Aldrin -- .045
4. Hept.Epox. -- .09
5. p,p'-DDE -- .09
6. Dieldrin -- .12
7. o,p'-DDT -- 0.24 ng
8. p,p'-DDD -- .24
9. p,p'-DDT -- .30
10. Dilan -- .75
11. Methoxychlor .60

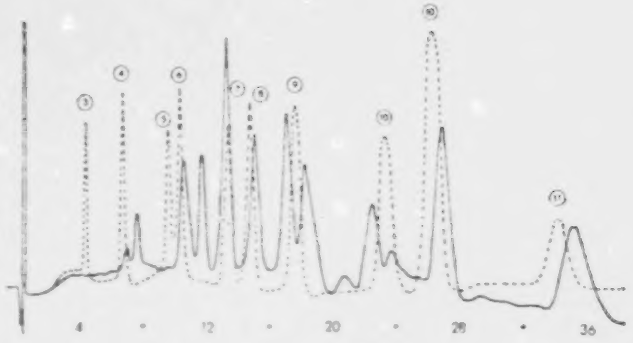
AROCLOR 1248
4 ng injection



AROCLOR 1254
3 ng injection



AROCLOR 1260
3 ng injection



Retention Values, Relative to Aldrin and Response Values, Relative to the Major Peak of Six of the Aroclor Compounds (poly-chlorinated biphenyls).

Column: Pyrex glass , 6-ft., 4 mm. i.d., 1.5% OV-17/1.95% QF-1, 200°C Column Temp. Carrier flow 60 ml/min.

Detector: Electron capture, ^3H, parallel plate, 210°C.

Misc.[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		RRR[2]	RPH[3]	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH
	RRR[2]	0.27	0.19	0.27	0.17								
Phosdrin	0.32	.34	.03	.33	.03								
		.40	.05	.40	.07								
		.43	.27	.43	.27								
Thimet	.50	*.49*	*1.00*	*.47*	*1.00*	0.48	0.34	0.48	0.08				
Diazinon	.63	.62	.05	.61	.38	.63	.41	.63	.34				
	*			.73	.20	.74	.22	.74	.15				
Heptachlor	*.83	.82	.04	.80	.85	*.82*	*1.00*	*.82*	*1.00*				
δ-BHC	.92	.91	.02	.90	.33	.90	.38	.92	.31				
				.95	.26	.97	.32	.98	.24				
Aldrin	1.00			1.03	.13	1.05	.23	1.06	.68	1.04	0.23		
	*			1.22	.20	1.24	.23	1.26	.62	1.24	.14		
				1.30	.19	1.33	.21	1.34	.56	1.34	.08		
M Parathion	1.45			1.44	.13	1.49	.15	1.50	.42	1.48	.07		
Hept. Epoxide	1.54			1.54	.27	1.57	.29	1.58	.96	1.56	.29		
Malathion	1.63									1.62	.33	1.61	0.09
Parathion	*1.81			1.83	.19	1.88	.21	1.88	.65	1.80	.58	1.78	.16
										1.97	.13		
p,p'-DDE	2.23			2.26	.03			2.21	.13	2.20	.19		
								2.32	.17	2.29	.36		
Dieldrin	2.40			2.43	.05	2.52	.03	2.50	.24	2.47	.68	2.53	.39
Endrin	2.93			2.78	.07	2.85	.04	2.85	.32	*2.82*	*1.00*	2.81	.42
o,p'-DDT	3.17			3.13	.02			3.18	.24	3.16	.53	*3.20*	*1.00*
p,p'-DDD	*3.49			3.57	.04	3.67	.02	3.66	.16	3.60	.50	3.50	.52
p,p'-DDT	4.18			4.04	.03			4.12	.05	4.08	.66	4.10	.74
Ethion	4.44									4.42	.08	4.38	.58
												5.0	.07
										5.28	.11	5.4	.38
Dilan I	5.7											5.7	.18
										5.95	.09		
Dilan II	6.4									6.4	.09	6.4	.00
Methoxychlor	8.3									8.4	.05	8.4	.43

[1] Relative retention ratios given for some common pesticides for comparison purposes.

[2] RRR - Means the retention relative to aldrin.

[3] RPH - Means the peak height response relative to that of the tallest peak shown in *italics*.

*Indicates chlordane peaks eluting in the appropriate area.

Retention Values, Relative to Aldrin and Response Values, Relative to the Major Peak of Six of the Aroclor Compounds (poly-chlorinated biphenyls)

Column: Pyrex glass, 6-ft., 4 mm. i.d., 4% SE-30/6% QF-1, 200°C Column temp.
Carrier flow 70 ml/min.

Detector: Electron capture, ^3H, parallel plate, 210°C.

Misc.[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		RRR[2]	RPH[3]	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRR	RPH
	RRR[2]	0.21	0.37	0.25	0.26								
Phosdrin	0.32	.32	.07	.32	.07								
	*	.34	.13	.35	.13	0.34	0.04						
		.39	.40	.39	.33	.38	.08						
2,4-D(ME)	.44	*.44*	*1.00*	*.48*	*1.00*	.43	.52	0.44	0.14				
Thimet	.47					.47	.03						
Simazine	.54	.55	.04	.55	.37	.53	.54	.55	.42				
Lindane	.60	.60	.05	.62	.28	.61	.38	.62	.25				
δ-BHC	.69	.73	.06	.72	.70	*.72*	*1.00*	.73	.99				
2,4-D(BE)	* .78			.79	.31	.77	.43	.80	.34				
Heptachlor	* .83			.82	.27	.81	.36	.83	.36				
Ronnell	.91			.90	.14	.89	.18	.90	.61	0.90	0.38		
Aldrin	1.00			1.02	.16	1.00	.22	1.03	.66	1.02	.25		
	*			1.09	.20	1.07	.27	1.10	.68	1.08	.14		
				1.16	.03	1.15	.02	1.18	.10				
M Parathion	1.33			1.31	.24	1.30	.30	*1.32*	*1.00*	1.29	.79	1.30	0.10
Hept. Epoxide	*1.43			1.48	.19	1.47	.23	1.50	.78	1.48	.95	1.52	.17
p,p'-DDE	1.82			1.77	.03	1.77	.03	1.80	.19	1.75	.53	1.80	.07
Captan	1.94			1.96	.04	1.90	.03	1.92	.25	1.87	.83	1.93	.08
o,p'-DDD	1.98					2.02	.03			2.00	.09	2.02	.36
Dieldrin	2.12									2.14	.28	2.18	.40
								2.24	.07	*2.21*	*1.00*		
o,p'-DDT	2.39			2.27	.05	2.27	.02	2.30	.27				
Endrin	2.42									2.51	.78		
p,p'-DDD	*2.55									2.62	.38	*2.58*	*1.00*
Thiodan II	2.72					2.69	.03	2.73	.18				
												2.81	.35
p,p'-DDT	3.12							3.12	.06	2.99	.91	3.10	.67
Trithion	3.20											3.39	.59
										3.55	.12	3.71	.05
												3.95	.42
Dilan I	4.40									4.20	.10		
Methoxychlor	4.6									4.66	.12	4.83	.31
Dilan II	5.1									5.54	.05	5.77	[illegible]
												6.2	.20

[1] Relative retention ratios given for some common pesticides for comparison purposes.

[2] RRR - Means the retention relative to aldrin.

[3] RPH - Means the peak height response relative to that of the tallest peak shown in *italics*.

*Indicates chlordane peaks eluting in the approximate area.

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Revised 6/77

Retention values, relative to aldrin and response values, relative to the major peak, of six of the Aroclor compounds (polychlorinated biphenyls).

Column: Pyrex glass, 5 ft., 5/32" i.d., 5% OV-210, 200°C column temp., carrier flow 45 ml/min.

Detector: Electron capture, ^3H, parallel plate, 205°C.

Miscellaneous[1] Pesticides		#1221		#1232		#1242		#1248		#1254		#1260	
		[2]RRR	[3]RPH	RRR	RPH	RRR	RPH	RRR	RPH	RRP	RPH	RRR	RPH
	[2]RRR	0.33	0.27	0.33	0.19								
		.41	.10	.42	.07								
		.46	.31	.46	.24	0.47	0.01						
		.51	*1.00*	*.52*	*1.00*	.53	.43	0.52	0.08				
		.61	.02	.62	.30	.62	.50	.60	.23				
Diazinon	0.73	.72	.06	.70	.20	.71	.22	.69	.11				
		.79	.08	.80	.84	*.81*	*1.00*	.80	.58				
		.88	.03	.88	.13	.88	.16	.86	.05				
β-BHC	.94	.92	.03	.93	.59	.94	.66	.92	.68	0.91	0.25		
				1.12	.29	1.12	.29	1.08	.54	1.08	.13		
1-Hydroxychlordene	1.30			1.29	.27	1.29	.17	1.28	.49	1.27	.38	1.28	0.05
				1.41	.54	1.41	.52	*1.37*	*1.00*	1.39	.26		
								1.47	.06	*1.46*	*1.00*	1.49	.20
				1.62	.15	1.62	.17	1.59	.24				
Hept. Epoxide	1.72							1.77	.10	1.77	.41	1.72	.04
Dimethoate	1.79			1.83	.03							1.86	.44
p,p'-DDE	1.81			1.96	.07	1.97	.04	1.92	.22	1.92	.75		
o,p'-DDT	2.26											2.06	.48
Malathion	2.32			2.32	.13	2.32	.08	2.28	.32	2.28	.77		
M. Parathion	2.44									2.40	.57	2.44	.89
										2.63	.11	2.66	.22
				2.82	.05	2.86	.04	2.78	.19	2.79	.20		
p,p'-DDT	3.29			2.99	.07			2.96	.08	2.93	.80	*2.93*	*1.00*
								3.60	.02	3.63	.21	3.58	.43
								4.32	.02	4.29	.13		
Methoxychlor	4.70											4.41	.76
										5.32	.06	5.38	.34
												6.5	.05
Dilan II	8.4											8.0	.10
	[4]RRA		.014		.0097		.02		.02		.025		.027

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[1] Relative retention ratios given for some common pesticides for comparison purposes.

[2] Indicates the retention ratio, relative to aldrin.

[3] Indicates the peak height response relative to that of the tallest peak shown in *italics*.

[4] Indicates the peak height response of the tallest peak relative to that obtained from an equivalent amount of aldrin.

Retention indices for all 210 possible chlorinated biphenyls on 13 GC liquid phases have been tabulated in the following reference:

> Identification of the Individual Polychlorinated Biphenyls in a Mixture by Gas-Liquid Chromatography, Albro, P. W., Haseman, J. K., Clemmer, T. A., and Corbett, B. J., J. Chromatogra., 136, 147 (1977).

DETERMINATION OF TCDD RESIDUES IN HUMAN MILK, BEEF LIVER, FISH, WATER, AND SEDIMENT

I. INTRODUCTION

The highly toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may be formed as a by-product in manufacturing processes utilizing tetrachlorobenzene to produce trichlorophenol. Under very basic, high temperature, high pressure conditions, 1,2,4,5-tetrachlorobenzene is hydrolyzed to the 2,4,5-trichlorophenate. Acidification yields the phenol. Unfortunately, a condensation can take place in this reaction resulting in formation of TCDD. Herbicides containing esters of 2,4,5-trichlophenoxyacetic acid (2,4,5-T) manufactured from trichlorophenol have been found to contain trace amounts of TCDD. TCDD has been recognized as an extremely toxic (oral LD_{50} 0.6 mg/kg, guinea pig), teratogenic compound that is stable in biological systems. The toxicological properties of TCDD have been well documented.

Because of its toxicity and occurrence as a trace contaminate in chemical products, it is necessary to analyze for TCDD at picogram/gram (low and sub-parts per trillion, ppt) levels, which are below the usual limits of detection for pesticide residue analysis. The analysis of human, biological, and environmental samples for possible TCDD contamination in the ppt concentration range is complicated by the presence of many interfering components ranging from naturally occurring compounds to industrial pollutants, such as PCBs, and the agricultural chemicals DDT, DDE, etc.

An extremely efficient and specific analytical cleanup procedure is a prerequisite for ppt TCDD analysis specifying GLC MS detection techniques. The GLC MS detection technique must be ultra sensitive and also highly specific because of the required low ppt detection limits. High resolution glass capillary column GLC interfaced with high resolution MS multiple ion selection analysis provides the required GLC resolution, MS sensitivity, and specificity for TCDD analysis in the 0.02-100 ppt concentration range.

Described below are the sample preparation procedures and capillary column GLC HRMS techniques developed and currently applied by EPA laboratories for isolation, detection, quantification, and confirmation of TCDD residues in human milk, beef liver, fish, water, and sediment extracts. The results of quality assurance samples and samples with unusual contamination are also discussed.

REFERENCE:

Sample Preparation Procedures and Gas Chromatography/Mass Spectroscopic Methods of Analysis for TCDD, Harless, R. L., Oswald, E. O., and Wilkinson, M. K., Analytical Chemistry Branch, U.S. EPA, HERL, ETD, Research Triangle Park, NC 27711. Submitted for publication to Anal. Chem.

II. PRINCIPLE:

Tissue, milk, water, soil, and sediment samples are subjected to an "acid-base" sample preparation procedure involving saponification with hot caustic followed by extraction with hexane, washing with concentrated sulfuric acid, cleanup by alumina column chromatography, and capillary column GLC/high resolution mass spectrometric multiple ion selection (GLC HRMS) analysis for TCDD residues. Fish tissue is subjected to a "neutral" cleanup procedure that is similar except that extraction is carried out with acetonitrile, and cleanup by solvent partitioning and Florisil column chromatography precedes the alumina column cleanup. ^{37}Cl-TCDD is added to all samples as an internal standard or marker to monitor and determine the analytical cleanup procedure efficiency.

III. SAFETY PRECAUTIONS:

TCDD is toxic and can pose grave health hazards if used improperly. Techniques for handling radioactive and infectious materials are applicable to TCDD. Only qualified individuals who are trained in laboratory procedures and familiar with the dangers of TCDD should handle this substance. Females of childbearing age should not work with this material.

A good laboratory practice involves routine physical examinations and blood checks of employees working with TCDD. Also, facial photographs using oblique photoflood lighting should be periodically taken to detect chloroacne, which is an early sign of overexposure.

IV. EQUIPMENT:

1. Gas chromatograph, Varian Model 2700, equipped with an SE-30 WCOT glass capillary column, 30 m x 0.25 mm, i.d. The capillary column yielded an efficiency of 113,000 effective plates measured at the ^{35}Cl-TCDD peak. Splitless injection incorporating n-tetradecane was employed.

2. Mass spectrometer, Varian 311A, interfaced to the chromatograph so as to ensure maximum transfer efficiency. The spectrometer was equipped with a turbo-molecular vacuum pumping system, combination chemical ionization (CI) and electron impact (EL)

ion source, and a Varian eight channel hardware (manual control) multiple ion selection (MIS) device. This vacuum system easily accommodated the 5 ml/minute helium flow from the GLC MS interface and did not contribute detectable background contamination. The MIS device was operated in the normal coupled electric mode (jumping the acceleration voltage). Each MIS channel was equipped with individual controls for selecting the acceleration voltage, measuring range, output signal bandwidth, compensation for background contamination, and intergration rate. The intensities of the selected masses were monitored in a time division multiplex system, setting alternately to each of the selected masses and recording their intensities simultaneously on an eight channel Soltec recorder. The adjustable integration rate, 0.01 second to 1 second was sufficient to accurately reproduce capillary column peaks two seconds wide at half height.

Alternative Instrumentation: Current MS instrumentation used by other laboratories (EPA contract laboratories) for TCDD analysis include (1) AEI MS-30, (2) AEI MS-50, and (3) Varian CH-5DF. These instruments are interfaced with a packed column gas chromatograph and use high resolution MS double ion monitoring techniques.

The general requirements for the GLS MS instrumentation are:

(1) Packed or preferably capillary column GLC introduction of the sample.

(2) High resolution (10,000 minimum) MS mass analysis.

(3) Ultra high sensitivity (1 to 50 pg TCDD quantification standards).

3. Pasteur pipets, 5.75 inches (14.6 cm) x 0.5 cm i.d.

4. Glass column, 50 cm x 11 mm, equipped with a Teflon stopcock and removal glass tip.

5. Desiccator, equipped with Drierite, which can accommodate adsorbent-packed Pasteur pipets.

6. Reflux condenser, water cooled, equipped with 100 ml boiling flasks.

7. Separatory funnels, 250 ml.

8. Evaporation apparatus including a 12 ml distillation receiver, micro-Snyder column Kontes K-569251, and steam bath.

9. Filter funnel.

10. Filter tube, glass, 16 cm x 42 mm.

11. Glass column, 39 cm x 11 mm i.d., with a 125 ml reservoir and Teflon stopcock.

12. Kuderna-Danish (K-D) evaporative concentrator, 250 ml.

13. Chromaflex sample tube, 2 ml, graduated, Kontes K-422560.

14. Glass tubing, 7 cm x 3 mm i.d.

15. Blender, Waring, or equivalent.

16. Magnetic stirrer.

17. Hotplate, explosion proof.

18. Mills type concentrator tube, Kontes K-570050.

V. REAGENTS:

1. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), ^{37}C labeled, isotopic purity >98% ^{37}Cl, Eco-Control, Inc., 71 Rogers St., Cambridge, MA 02142.

2. TCDD analytical standard, Dow Chemical Co., Midland, MI; ITT Research Institute, Chicago, IL; and Eco-Control, Inc.

3. Hexane, acetone, benzene, methylene chloride, ethyl alcohol, acetonitrile, Mallinckrodt Nanograde.

4. Carbon tetrachloride, Fisher ACS grade, 0.01% water maximum; a greater water content can cause TCDD to elute in the incorrect fraction.

5. Alumina, neutral, activity grade 1, Woelm.

6. Florisil, 60-120 mesh, suitable for pesticide residue analysis by the criteria in Section 3,D, activated at 225°C for 24 hours before use.

7. Sodium carbonate, sodium sulfate, and ammonium chloride, Mallinckrodt AR grade, Soxhlet extracted overnight with methylene chloride and dried at 200°C.

8. Potassium hydroxide and sulfuric acid, Mallinckrodt AR grade.

9. Glass wool, pre-extracted with methylene chloride.

10. Water, passed through a column of activated carbon and distilled.

11. Nitrogen for solvent evaporation, Zero Grade, Liquid Air, Inc., New Orleans, LA.

12. Dry ice.

VI. PREPARATION OF CHROMATOGRAPHIC CLEANUP COLUMNS:

1. Alumina

   a. Prewash and dry a disposable Pasteur pipet and plug the tip with glass wool.

   b. Pack the pipet with 4.5 cm of neutral alumina and top the column with 0.5 cm of anhydrous, granular sodium sulfate.

   c. Wash the column with 4 ml of methylene chloride and force the residual solvent from the column with a stream of dry nitrogen.

   d. Store the prepared columns in an oven at 225°C at least 24 hours.

   e. Before use, equilibrate the oven-activated columns to room temperature in a desiccator over Drierite.

2. Florisil

   a. Pack a 500 x 11 mm glass column with 15 grams of activated Florisil.

   b. Pack a 2.5 cm layer of anhydrous sodium sulfate on top of the Florisil.

   c. Hold at 225°C until ready for use (a minimum of 24 hours).

   d. Cool the column to near room temperature and prewash with 100 ml of hexane.

VII. SAMPLE PREPARATION AND CLEANUP - ACID-BASE PROCEDURE:

1. Lean Tissue

   a. Grind tissue samples to obtain a homogeneous sample.

b. Weigh a 10-20 gram sample into a 100 ml boiling flask and add 20 ml of ethyl alcohol and 40 ml of 45% KOH solution.

c. Add 5-10 ng of ^{37}Cl-TCDD standard solution.

d. Attach the flask to a water cooled reflux condenser and heat under reflux with stirring for 2.5 hours.

e. Cool and transfer the solution to a 250 ml separatory funnel.

f. Rinse the boiling flask with 10 ml of ethyl alcohol followed by 20 ml of hexane, and add to the separatory funnel.

g. Extract the solution with four 25 ml portions of hexane and combine the hexane extracts.

2. Adipose Tissue

a. Grind or render adipose samples, if necessary, to obtain a representative sample free of connective or other tissue.

b. Add 5-10 ng of ^{37}Cl-TCDD to 10 grams of sample.

c. Add 15 ml of distilled water to the sample. Extract and continue as described in Subsection 1, b-g, for lean tissue.

3. Milk

a. Add 2.5 ng of ^{37}Cl-TCDD standard solution to 10-20 grams of milk.

b. Extract the sample as described in Subsection 1, b-g, for lean tissue.

4. Water

a. Fortify one kilogram of a well mixed water sample (including particulate matter, if present) with 2.5 ng of ^{37}Cl-TCDD standard solution.

b. Extract the sample with three 100 ml portions of methylene chloride.

c. Evaporate the combined extracts to near dryness in a K-D concentrator with an attached Snyder column utilizing a steam bath. Complete the evaporation to dryness by placing the tube in a warm water bath under a gentle stream of dry nitrogen.

d. Transfer the residue to a separatory funnel with several rinsings of hexane totaling 100 ml.

e. Wash the hexane solution with 50 ml of 1 N KOH solution followed by concentrated H_2SO_4 as described in Subsection 6 on cleanup.

5. Soil and Sediment

a. Fortify 10-20 grams of well mixed sample with 2.5 ng of ^{37}Cl-TCDD.

b. Extract as described in Subsection 1, b-g, for lean tissue.

c. After refluxing and cooling, decant the solution into a separatory funnel through a filter funnel packed with glass wool.

d. Rinse the boiling flask and filter funnel with two 10 ml portions of ethyl alcohol followed by 20 ml of hexane.

e. Extract the combined solution with four 25 ml portions of hexane that had previously been used to rinse the boiling flask and filter funnel.

6. Cleanup

a. Wash the combined hexane extracts, obtained as describe above in Subsections 1-5, with 25 ml of 1 N KOH solution followed by four 50 ml portions of concentrated H_2SO_4.

b. Add 25 ml of water and shake. Neutralize the water and hexane layers by addition of powered Na_2CO_3 in small portions with mixing until CO_2 evolution ceases.

c. Discard the aqueous layer, and dry the hexane layer by passage through the 39 cm x 11 mm i.d. glass column containing 10 cm of anhydrous powered Na_2CO_3.

d. Transfer the hexane concentrate to an alumina column, prepared as described in Subsection VI, 1, that was prewetted with one ml of hexane.

e. Wash the column with 6 ml of CCl_4 and discard the wash.

f. Elute the column with 4 ml of methylene chloride and collect in a 21 ml distillation receiver.

g. Cap the distillation receiver with a micro-Snyder column and evaporate the methylene chloride just to dryness on a hot water or steam bath.

h. Add two separate 2 ml portions of hexane to the distillation receiver and evaporate each just to dryness.

i. Dissolve the residue in 3 ml of hexane and chromatograph on a second alumina column as just described.

j. Evaporate the methylene chloride eluate from the second column just to dryness.

k. Add 2 ml of benzene to the receiver and concentrate to ca 100 μl.

l. Transfer the benzene solution quantitatively to a 2 ml graduated Chromaflex sample tube.

m. Carefully concentrate the benzene ca 100 μl under a gentle stream of dry nitrogen. Quantitatively transfer with two 25 μl benzene rinses to a glass tube (7 cm x 3 mm i.d.) that is sealed at one end.

n. Carefully concentrate the extract to 60 μl and flame seal the tube. Store below 0°C until analysis by GLC MS.

VIII. SAMPLE PREPARATION AND CLEANUP - NEUTRAL PROCEDURE:

1. Extraction of Fish Tissue

a. Grind fish tissue to obtain a homogeneous sample.

b. Place a 15 gram sample and 150 grams of anhydrous granular sodium sulfate in a blender jar and blend for one minute.

c. Blend next with 50 gram portions of dry ice until the sample is thoroughly powered.

d. Transfer the powder to an Erlenmeyer flask and add 10 ng of ^{37}Cl-TCDD directly onto the powder.

e. Rinse the blender jar with acetonitrile.

f. Add the rinsings plus enough additional acetonitrile to the flask to make a total of exactly 150 ml.

g. Mix vigorously on a magnetic stirrer for 2 hours.

h. Filter the mixture through a glass filter tube containing 30 grams of anhydrous granular sodium sulfate.

2. Cleanup

   a. Partition exactly a 100 ml aliquot of the acetonitrile extract, representing 10 grams of the original sample, with 50 ml hexane that is saturated with acetonitrile. Draw the acetonitrile (bottom) layer into a 500 ml separatory funnel.

   b. Partition the hexane layer with two 100 ml portions of acetonitrile saturated with hexane followed by one 50 ml portion of the same solvent and combine with the above acetonitrile (350 ml total volume).

   c. Partition the combined acetonitrile layers with 10 ml of hexane saturated with acetonitrile.

   d. Draw the acetonitrile layer into a 500 ml flat bottom 20/40 ₮ Florence flask and concentrate to ca 10 ml under a Snyder column on an explosion-proof hotplate.

   e. Transfer the concentrate by repeated rinsings with a total of 200 ml of hexane to a K-D apparatus with a 10 ml Mills tube attached. Concentrate each of 5-10 ml on a hot water or steam bath.

   f. Transfer the hexane concentrate to a Florisil column, prepared as described in Section VI,2, using three 5 ml portions of hexane.

   g. Elute the column with 100 ml of hexane-methylene chloride (90:10 v/v) and discard this eluate.

   h. Elute with 100 ml of hexane-methylene chloride (75:25 v/v) and collect in a Kuderna-Danish evaporator equipped with a 100 ml Mills tube.

   i. Concentrate to ca 3 ml on a hot water or steam bath.

   j. Dissolve the concentrate in 100 ml of hexane and again evaporate to ca 3 ml.

   k. Transfer the concentrate to an alumina column and proceed with chromatography as described in the acid/base procedure (Subsection VII,6), but use only one alumina column.

IX. CAPILLARY COLUMN GC/HRMS MULTIPLE ION SELECTION ANALYSIS:

1. Tune the magnet current to perfluorokerosene (PFK) m/e 318.9793 and adjust the spectrometer from 5000 to 9000 mass resolution.

2. Monitor the ESA voltage and use in calculating the exact acceleration voltage required for the masses m/e 327.8847 $C_{12}H_4O_2{}^{37}Cl_4$, m/e 321.8936 $C_{12}H_4O_2{}^{35}Cl_3{}^{37}Cl$, and m/e 319.8965 $C_{12}H_4O_2{}^{35}Cl_4$.

3. Introduce the calculated values of MIS channels 2, 3, and 4.

4. Inject 2 µl of TCDD quantification standard, 500 pg/µl ^{37}Cl-TCDD (labeled 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, ^{37}Cl, isotopic purity greater than 98%) and one pg/µl TDCC, and 0.5 µl of *n*-tetradecane (keeper) into the capillary column (on-column splitless injection) maintained at 80°C.

5. Rapidly turn the GLC oven manual temperature control to 265°C exactly 6 minutes after injection of the sample; the manual control provides an accurate and rapid heating rate of 34°C/minute.

6. Close the solvent vent valve exactly 14 minutes after injection of the sample.

7. Optimize the MS sensitivity for the source operating pressure, 6×10^{-6} Toor, using PFK m/e 318.9793.

8. Initiate the MIS analysis 16 minutes after injection of the sample.

9. Adhering to a strict (stopwatch) time schedule of events, the GLC HRMS experimental retention time for TCDD was 23 minutes ± 15 seconds with the following GLC and MS parameters:

   30 m SE-30 WCOT glass capillary column
   injection port temperature, 260°C
   GLC transfer line into the MS ion source, 255°C
   ion source temperature, 240°C
   variable acceleration voltage 3 kV maximum
   electron energy, 70 eV
   filament emission, 1 mA
   mass resolution 5,000-10,000
   multiplier gain, greater than 10^6

X. COCl LOSS ANALYSIS:

1. Tune the magnet current to PFK m/e 254.9856.

2. Introduce the exact acceleration voltages required for TCDD masses m/e 256.9327, m/e 258.9298, m/e 319.8965, m/e 321.8936, and m/e 327.8847 into respective MIS channels (M^+—COCl peak, m/e 258.9298, is used to confirm TCDD structure).

3. Perform the analysis, adhering to the previously described time schedule of events.

4. Observe the GLC HRMS five channel simultaneous response for ^{37}Cl-TCDD and TCDD and record at the correct GLC retention time for TCDD.

XI. ELEMENTAL COMPOSITION ANALYSIS:

1. Adjust the mass spectrometer for 10,000 mass resolution using PFK m/e 318.9793 as reference.

2. Initiate the peak matching analysis, adhering to the exact time schedule of events utilized in the GLC HRMS MIS analyses.

3. Display alternately the reference mass and the exact mass range of interest and view simultaneously on the MS oscilloscope.

XII. DETECTION AND RECOVERY RESULTS AND DISCUSSION OF METHODS:

1. Glass capillary columns enhanced the GLC HRMS method of analysis because (a) they provided the required resolution of complex mixtures into individual components before they entered the mass spectrometer; (b) the narrow band width of the TCDD component enhanced MS sensitivity; (c) direct coupling of the capillary column to the mass spectrometer ensured maximum transfer efficiency; (d) capillary column bleed rate was low, therefore background contamination was minimized and MS sensitivity was enhanced.

2. The requirements imposed on the mass spectrometer used as a GLC detector in these analyses were (a) extremely stable electronic circuits; (b) ultra high sensitivity; (c) specific mass detection. The requirements were satisfied by optimizing all components that influenced sensitivity, noise, and mass resolution. The MIS response for a quantification standard, ^{37}Cl-TCDD and TCDD, is shown in Figure 1.

3. Precision and Accuracy of GLC HRMS Technique

When adjusted for 7500 mass resolution and used as a GLC detector in MIS analyses, the mass spectrometer will give positive responses for those components eluting from the gas chromatograph that yield a molecular or fragment ion in the range + 30 millimass units of TCDD masses m/e 319.8965, m/e 321.8936, and m/e 327.8847. This minimizes the interference from contaminating components, thus yielding responses that are reproducible and linear relative to the amount of TCDD injected. Two concentration ranges, 0.2-2 pg and 2-10 pg, were used to provide the most efficient and accurate quantification of TCDD because of the extremely high sensitivity and manual control attenuation used in these analyses. Sample extracts should be diluted or concentrated as required for quantification purposes. The reproducibility of peak height response for TCDD standards, 1 pg/μl or 5 pg/μl, during daily operation was ± 20%. The TCDD m/e 320 and m/e 322 chlorine isotope ratio ranged from 0.75-0.95 to 1. The variation from the theoretical chlorine ratio, 0.8 to 1.0, was attributed to the MIS integration rate, very narrow capillary column GLC peaks, and the small amount of TCDD being analyzed.

The GLC HRMS peak matching accuracy for known elemental compositions was determined to be ± 2 millimass units at 9500 mass resolution with PFK as reference. The reference mass and TCDD mass were observed to be exactly superimposed on the mass spectrometer oscilloscope at the exact GLC HRMS retention time of TCDD.

4. Recovery of ^{37}Cl-TCDD and Measurement of TCDD

The MIS simultaneous peak height responses (m/e 328, m/e 322, and m/e 320) of sample and sample fortified with a known amount of ^{37}Cl-TCDD and TCDD were used to determine the sample preparation procedure efficiency (percentage recovery), TCDD residue level, and limit of detection. The criteria utilized for confirmation of TCDD are shown in Table 1. The percentage recovery experimental value was used to correct the TCDD residue level and limit of detection for ^{37}Cl-TCDD recovery losses. A minimum acceptable percentage recovery (50%) was established for reporting TCDD analyses. The infrequent analyses exhibiting less than 50% recovery were discarded. TCDD results were not corrected for recovery values greater than 100%. Recovery values between 100 and 135% were attributed to interference from PCBs and unidentified contamination. Due to the widespread distribution of PCBs, the accuracy of ^{37}Cl-TCDD determination primarily depends on the sample preparation procedure efficiency and specificity, and the capillary column GLC resolution of components. The MS mass resolution, ca 45000, required to separate ^{37}Cl-TCDD m/e 327.8847 and PCB m/e 327.8758 is not feasible owing to MS limitations. For occasional and highly contaminated sample extracts, the ^{37}Cl-TCDD m/e 328 peak height was determined utilizing the PCB m/e 326 peak height to calculate the PCB contribution to the m/e 328, a mixture of ^{37}Cl-TCDD and PCB.

5. Limit of Detection

The limit of detection was defined as the quantity of TCDD that would provide a signal to noise ratio greater than 2.5:1 with clearly defined peak shapes (m/e 320 and m/e 322) in the proper isotopic ratio. The limit of detection varied from sample to sample because of percentage recovery, sample size, matrix effects, and electronic noise present in the time frame of measurements.

6. Isotopic Purity of ^{37}Cl-TCDD Fortification Standard

The ^{37}Cl-TCDD standard, 1 ng/µl in benzene, was subjected to MIS analyses for determination of purity and possible interferences for sub-ppt TCDD analyses prior to human milk studies. A TCDD isomer was detected at 1 pg/ng ^{37}Cl-TCDD. The GLC HRMS peak matching technique with PFK as reference was used to confirm the elemental composition of m/e 319.8965 and 321.8936, both corresponding to TCDD, in a concentrated solution of ^{37}Cl-TCDD standard. The elemental compositions, the m/e 320/322 Cl ratio, and the GLC retention time of ^{37}Cl-TCDD fortified with 2,3,7,8-TCDD were criteria used to confirm the presence of a TCDD isomer in ^{37}Cl-TCDD standard that satisfies the analytical criteria for 2,3,7,8-TCDD. These results indicated that 10 ng ^{37}Cl-TCDD foritification levels in 10 gram samples would produce 1 ppt TCDD analyses. This was confirmed experimentally. The fortification level was reduced from 10 to 2.5 ng ^{37}Cl-TCDD per sample to avoid false positive results in 0 to 2 ppt TCDD analyses.

7. Human Milk Studies

EPA has initiated a study to determine the possible presence of TCDD in human milk. Sample locations were selected based on the aerial application of 2,4,5-T for conifer release as part of a forestry management program. The samples were subjected to the described acid-base extraction and cleanup procedure prior to GLC HRMS MIS analysis.

The 60 µl human milk extracts were quantitatively concentrated to 7-20 µl using dry nitrogen gas for sub-ppt TCDD analysis. The MIS analysis sequence was sample, sample fortified with

^{37}Cl-TCDD, and TCDD. A typical analysis for TCDD residues in a QC sample of human mother's milk is shown in Figure 2. The corrected experimental results indicated the sample contained 1.2 ppt TCDD residue. This 10 gram sample had been fortified with 10 pg of TCDD, which corresponds to 1 ppt TCDD. The total TCDD analyses, analytical cleanup efficiency, TCDD residue level, and limit of detection were performed on injections of a sample and fortified sample. Duplicate analyses were usually performed on each sample to establish precision values.

The results of a quality assurance study incorporating human milk fortified with 2.5 ng ^{37}Cl-TCDD and 0-5 ppt TCDD are shown in Table 2. Evaluation of the experimental results and theoretical results after completion of study indicate: (a) the analytical cleanup procedure and MIS method of analysis provided reasonably accurate TCDD analysis in the 0.2-5 ppt concentration range; (b) false positive results were not detected; (c) 2.5 ng ^{37}Cl-TCDD fortification levels were adequate for recovery purposes; (d) the small amount, (2.5 pg) of TCDD, in adverse effects in 0-5 ppt TCDD analyses, with a 0.2 ppt detection limit. A representative number of positive results generated at this level of detection should be confirmed with supplemental techniques such as COCl loss, peak matching analyses, etc.

Contamination was a constant problem in 0-5 ppt TCDD analysis. A very efficient and optimized capillary column was required to resolve TCDD from contamination, and its effectiveness could be destroyed in the presence of high amounts of contamination. In general, PCBs were the contaminants of major concern. The mass resolutions 12,476 and 45,539 required to separate PCB masses 321.8677 and 327.8758 from TCDD masses 321.8935 and 327.8847 could not be used in 0-5 ppt TCDD analysis because of instrument design and sensitivity. The PCB interference to TCDD analysis was observed to have the following effects: (a) recovery of ^{37}Cl-TCDD became greater than 100% and (b) the TCDD m/e 320/322 chlorine isotope ratio was destroyed. Mass resolution of 5,000 to 8,000 was sufficient to resolve TCDD from other contamination present.

8. Fish Analysis

Edible portions (2.5 to 10 grams) of fish samples were fortified with 2.5 to 10 ng ^{37}Cl-TCDD and subjected to the described acid/base extraction and cleanup procedures. An MIS analysis is shown in Figure 3, for sample and fortified sample. Unusual and high concentrations of contaminate masses were detected at m/e 320 and m/e 322 in fish collected from polluted waters. The contamination was not detected in analyses of ocean perch, fish from specific locations, and beef liver during the analysis sequence. The high concentration of co-extractable components in fish caused serious problems (capillary column overload, co-elution of components, and decreased MS sensitivity). To minimize or cancel these effects, very high MS sensitivity (4-9 pg quantification standard) and small sample size (0.5 to 3 μl from 55 μl equivalent to a 10 gram sample) were used in analysis of fish.

A small number of highly contaminated fish extracts were subjected to additional GLC HRMS analyses and to a "neutral" cleanup procedure to confirm the presence of TCDD:

(a) MIS simultaneous response for the molecular ion cluster m/e 320, m/e 322, and m/e 324 to confirm the tetrachloro isotope ratio.

(b) MIS simultaneous response for m/e 320, m/e 322, m/e 257, and m/e 259 to confirm the M^+-COCl loss indicative of the TCDD structure.

(c) GLC HRMS peak matching analysis to confirm the elemental composition of the TCDD molecular ion, m/e 319.8965.

Two exact masses corresponding to TCDD isomers were observed eluting before and after TCDD. Contaminant masses, differing from the exact mass of TCDD, were also observed during the time frame of the analysis. Highly contaminated fish samples were subjected to a "neutral" cleanup procedure described in Section 9G. Capillary column GLC HRMS MIS analysis yielded positive ^{37}Cl-TCDD and TCDD responses essentially free of contamination. The quality assurance sample results utilized in these studies are shown in Table 3.

9. Water and Sediment Analysis

Water and sediment samples were collected from specific areas of the United States and subjected to the described analytical extraction and cleanup procedures prior to MIS analysis. The analytical results for quality assurance samples incorporated in these studies are shown in Table 4.

Evaluation of the results shown in Table 4 indicates that the analytical extraction and cleanup procedure and MIS technique provided reasonably accurate analysis for 10-1000 parts per quadrillion (10^{-15}) TCDD in water and 0-35 ppt TCDD in sediment. Water extracts were very clean. Significant amounts of contamination differing from the exact mass of TCDD were detected in specific sediment samples but did not interfere with TCDD analysis.

10. TCDD Isomers

The toxicological properties of TCDD isomers are known to be significantly different. The mass spectra of known TCDD isomers are identical except in the low mass range, and this minor difference would not be of significant value in ppt analysis of environmental or biological extracts. Therefore, it is extremely important that the gas chromatograph be equipped with high resolution capillary columns to resolve TCDD isomers before they enter the mass spectrometer. The 2,3,7,8-, 2,3,6,8-, and 1,2,3,4-TCDDs and a mixture consisting of 70% 1,3,6,8-TCDD and 30% of an unknown TCDD isomer have been separated in this laboratory using glass capillary column GLC HRMS. The SE-30 WCOT glass capillary column resolution of TCDD isomers and order of elution were similar to separations reported in the literature on an OV-101 glass capillary column. The 2,3,6,8-TCDD isomer was only partially resolved from 1,2,3,4-TCDD.

Several TCDD isomers have been detected and confirmed in environmental, biological, and chemical formulation samples using the described capillary column GLC HRMS techniques and coinjection of specific TCDD isomers.

Preliminary studies using the acid-base cleanup procedure and nanogram quantities of hexa-, hepta-, and octa-substituted dioxins (analytical standards) suggest that tetrachlorodioxin isomers are not formed from the degradation of higher chlorinated dioxins by acid-base sample preparation conditions. Nanogram quantities of 2,4,5-trichlorophenol showed no evidence of condensation to 2,3,7,8-TCDD under the same acid-base conditions.

XIII. ANALYTICAL QUALITY CONTROL:

The analytical cleanup laboratory should assign identification numbers to all samples. The samples and QC samples are fortified with 2.5-10 ng of ^{37}Cl-TCDD. The QC samples are fortified with 0 to 1, 250 pg (p to 125 ppt) of TCDD before extraction and cleanup. A method blank is included as part of the QC sample package. All sample extracts and quantification standards, ^{37}Cl-TCDD and TCDD, are submitted to the GLC MS laboratory in a blind fashion, i.e., there should be no way to distinguish QC and actual samples.

The efficiency, accuracy, precision, and validity of ppt TCDD analyses depend on an incorporated quality assurance program. The supplemental and conclusive GLC HRMS validation techniques involving analyses for M^{+}-COCl loss and GLC HRMS peak matching analysis (real time) can not easily be applied to 0-30 ppt TCDD analyses at this date, using the described procedures. Based on the incorporated quality assurance program, analytical criteria, GLC HRMS techniques, and multiple laboratory participation, the described methodologies have been shown to be effective for isolation, detection, and quantification of 0.02-100 ppt levels of TCDD in specific types of samples. Samples containing high ppt or part per billing (ppb) levels of TCDD can cause serious contamination problems in the sample preparation laboratories, which result in erroneous low ppt TCDD analysis of the sample next in the series. Extreme care and very clean laboratory practices are mandatory for low ppt TCDD analyses.

Results of some quality assurance studies are presented in Subsection XII.

TABLE 1. CRITERIA USED FOR CONFIRMATION OF 2,3,7,8-TCDD RESIDUES IN HUMAN, ENVIRONMENTAL AND FISH SAMPLES

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1. Capillary column GLC HRMS retention time of 2,3,7,8-TCDD.
2. Co-injection of sample fortified with ^{37}Cl-TCDD and TCDD standard.
3. Molecular ion chlorine isotope ratio (m/e 320 and m/e 322).
4. Capillary column GLC HRMS multiple ion monitoring response for TCDD masses (simultaneous response for elemental composition of m/e 320, m/e 322, and m/e 328, ^{37}Cl-TCDD).
5. Response of m/e 320 and m/e 322 greater than 2.5 times the noise level.

TABLE 2. ANALYTICAL RESULTS FOR 2,3,7,8-TCDD RESIDUES IN QUALITY ASSURANCE SAMPLES OF HUMAN MILK

Experimental Results			TCDD Fortification Level	
^{37}Cl-TCDD % Recovery	TCDD Detection Limit* (ppt)	TCDD Detected* (ppt)	(pg)	(ppt)
50	0.3	1.9	10	1.0
72	0.2	0.6	3	0.3
68	0.1	0.2	1	0.1
64	0.3	ND	0	0
68	0.4	0.9	9	0.9
84	0.2	1.4	20	2.0
64	0.2	0.4	5	0.5
51	0.2	ND	2	0.2
73	0.4	0.6	7.5	0.75
52	0.3	1.4	6.5	0.65
72	0.5	3.0	30	3.0
100	0.5	4.0	50	5.0
50**	0.2	ND	0	0

Each 10 gram sample was fortified with 2.5 ng ^{37}Cl-TCDD.

* Corrected for recovery.
** Method blank.
ND = Not detected.
ppt = Parts per trillion.

TABLE 3. SUMMARY OF ANALYTICAL RESULTS FOR QUALITY ASSURANCE SAMPLES (OCEAN PERCH, LAKE TROUT, BEEF LIVER) GENERATED DURING ANALYSIS OF FISH FOR TCDD RESIDUES

Experimental Results						
Sample Weight (gram)	^{37}Cl-TCDD Fortification Level (ng)	^{37}Cl-TCDD % Recovery	^{35}Cl-TCDD Detection Limit (ppt)*	TCDD Detected (ppt)*	TCDD Fortification Level (pg)	(ppt)
5 (1)	5	62	2	20	110	22
5 (1)	5	52	4	34	185	35
5 (1)	5	82	3	ND	0	0
5 (4)	5	100	3	ND	0	0
5 (3)	5	54	1	19	70	14
5 (3)	5	100	2	ND	0	0
5 (3)	5	78	2	ND	0	0
5 (1)	5	92	2	19	55	11
5 (1)	5	97	5	45	240	48
10 (1)	10	100+	1	8	130	13
10 (1)	10	100+	4	43	600	60
10 (2)	10	100+	7	ND	0	0
10 (2)	10	100+	3	ND	0	0
10 (1)	10	100+	4	76	1250	125
10 (4)	10	67	1	ND	0	0
10 (1)	5	93	3	56	650	65
10 (1)	10	84	4	73	620	62

* Corrected for recovery (1) ocean perch (2) lake trout (3) beef liver (4) method blank

ND = Not detected.

TABLE 4. SUMMARY OF ANALYTICAL RESULTS FOR QUALITY ASSURANCE SAMPLES GENERATED DURING ANALYSIS OF WATER AND SEDIMENT FOR 2,3,7,8-TCDD RESIDUES

Experimental Results						
Sample Type	Sample Weight (gram)	^{37}Cl-TCDD Fortification Level (ng)	^{37}Cl-TCDD Recovery	Detection Limit*	TCDD Detected*	TCDD Fortification Level
water	1000	2.5	73	15 ppqd	85 ppqd	75 ppqd
water	1000	2.5	90	50 "	730 "	1000 "
water	1000	2.5	66	14 "	ND	10 "
water	1000	2.5	95	10 "	50 "	50 "
water	1000	2.5	78	15 "	116 "	100 "
water	1000	2.5	78	15 "	28 "	25 "
water	1000	2.5	100+	41 "	422 "	500 "
sediment	50	2.5	69	0.13 ppt	1.0 ppt	1.0 ppt
sediment	50	2.5	100+	0.14 "	1.0 "	1.4 "
sediment	10	2.5	100+	0.6 "	2.5 "	1.6 "
sediment	10	2.5	96	0.5 "	3.3 "	4.6 "
sediment	10	2.5	100+	2.0 "	23.0 "	17.0 "
sediment	10	2.5	68	4.0 "	30.0 "	35.0 "
sediment	10	2.5	100	0.7 "	ND	0 "

ppqd = Parts per quadrillion (10^{-15}).

ppt = Parts per trillion (10^{-12}).

* Corrected for recovery.

ND = Not detected.

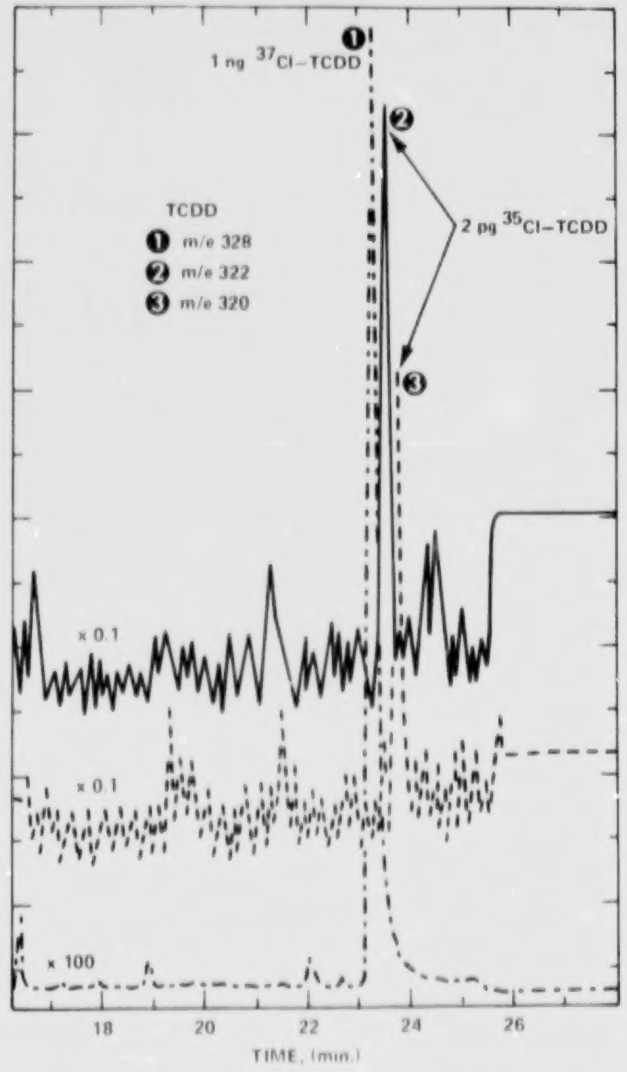

FIGURE 1. GLC/HRMS multiple ion selection response for 1 ng ^{37}Cl-TCDD and 2 pg ^{35}Cl-TCDD.

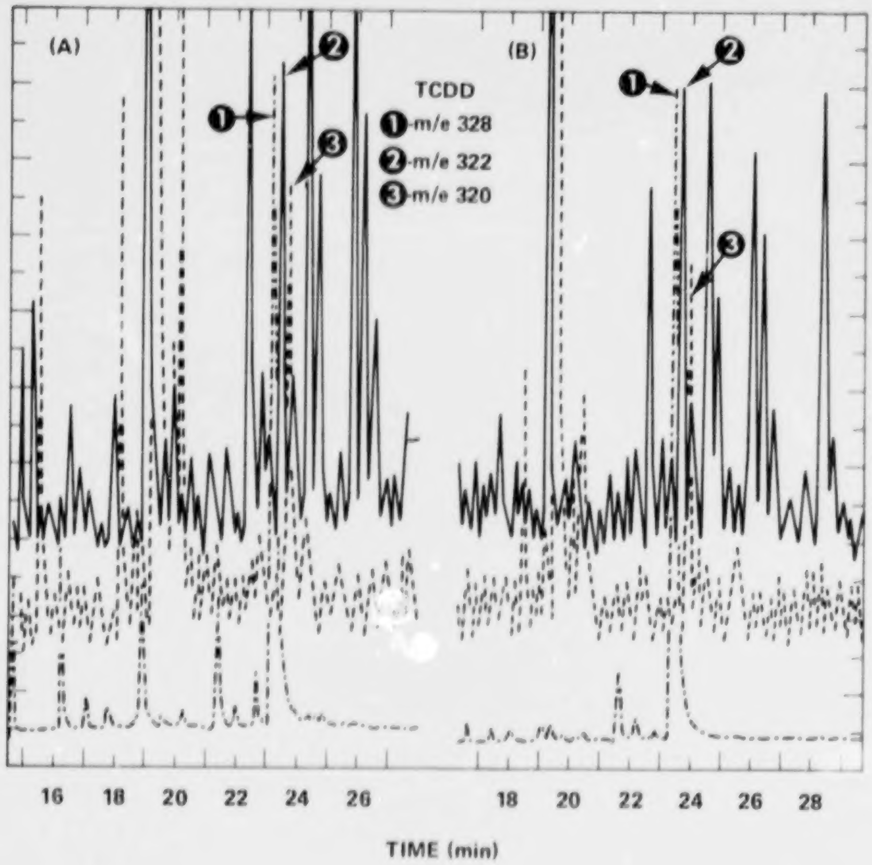

FIGURE 2. GLC/HRMS MIS monitoring analysis for TCDD residues in a human milk QA sample. (A) 2 μl of sample (fortified with 1 ppt TCDD). (B) 1 μl of sample fortified with 500 pg ^{37}Cl-TCDD and 1 pg ^{35}Cl-TCDD.

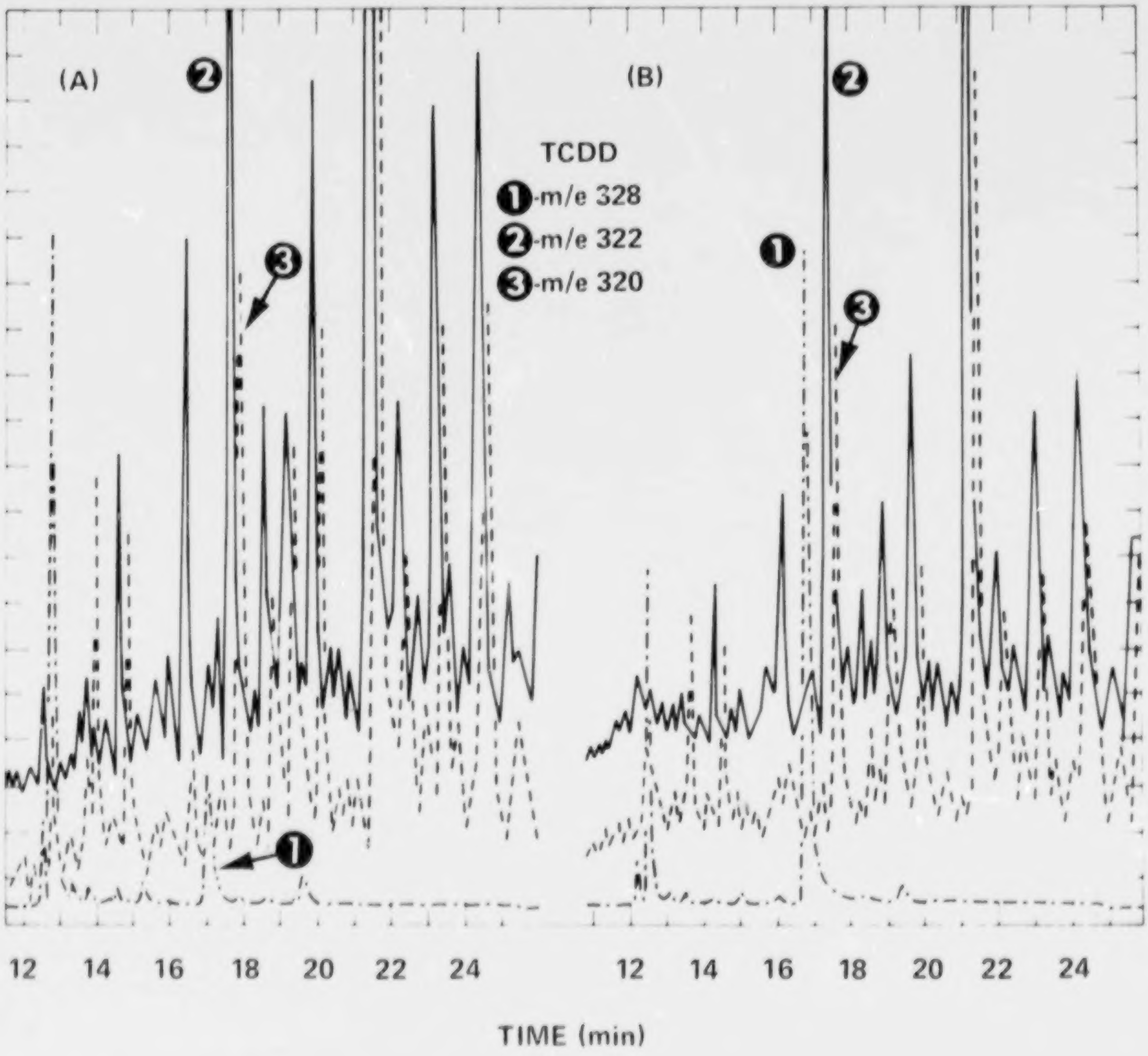

FIGURE 3. GLC/HRMS MIS monitoring analysis of a fish extract. (A) Sample 1 1 from 50 µl; (B) Fortified sample, 0.5 µl from 50 1 plus quantification standard.

THE SAMPLING AND ANALYSIS OF WATER FOR PESTICIDES

I. INTRODUCTION:

The methodology for the analysis of water described in this section was researched by Thompson et al. at the Environmental Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC (1). It is based on modification of the multiclass, multiresidue procedure for pesticides in air reported by Sherma and Shafik in an earlier paper (2). Recovery studies were conducted on 42 halogenated compounds, 38 organophosphorus compounds, and 7 carbamates, and the procedure proved acceptable (>80% recovery) for 58 of the 87 compounds tested. Thirteen compounds yielded recoveries exceeding 60%, while the remaining 16 compounds were recovered at levels below 60%. Concentration levels ranged from 0.09-400 ppb.

The present method provides the analyst with the means of simultaneously monitoring water samples for a wide variety of different pesticides, a capability not demonstrated for the few previously published under multiclass, multiresidue analytical procedures. For example, the method in the 1974 revision of this Manual included a florisil cleanup column and was tested with only 16 organochlorine and 9 less-polor organophosphorus pesticides. Other published multiclass GLC methods have employed cleanup on silica gel, Florisil, and alumina or no column cleanup. None of these is as broadly applicable as the following method.

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2. A Multiclass, Multiresidue Analytical Method for Pesticide Residues in Air, Sherma, J., and Shafik, T. M., Arch. Environ. Contam. Toxicol. 3, 55 (1975). (See Section 8, this Manual).

3. Persistence of Pesticides in River Water, Eichelberger, J. W. and Lichtenberg, J. J., Environ. Sci. and Technol. 5(6), 541 (1971) (Table 1).

4. Pesticide Residue Analysis in Water--Training Manual PB-238 072, U.S. Environmental Protection Agency, OWPO, National Training Center, Cincinnati, Ohio, September, 1974, distributed by the National Technical Information Service, U.S. Department of Commerce.

5. Gas Chromatographic Determination of Residues of Methyl Carbamate Insecticides in Crops as their 2,4-Dinitrophenyl Ether Derivatives, Holden, E. R., J. Ass. Offic. Anal. Chem. 56, 713 (1973) and 58, 562 (1975).

II. PRINCIPLE: (See Schemes I and II, Pages 15 and 16)

Compounds are extracted from water with methylene chloride, and the extract volume is reduced at low pressure and temperature in an evaporative concentrator. Compounds are separated into groups on a column of deactivated silica gel by elution with solvents of increasing polarity. Organochlorine compounds are determined by gas chromatography with an electron capture detector, organophosphorus compounds with a flame photometric detector, and carbamates by electron capture GLC after conversion to 2,4-dinitrophenyl ether derivatives.

III. GRAB SAMPLE COLLECTION:

The sampling location and the method of drawing the sample will, to a great extent, be dictated by the objectives of the sample data. If the objective is to determine the highest pesticide pollution present in a stream or lake, a grab sample might be drawn at the point of highest polution introduction. If, on the other hand, the objective is an average residue profile of the entire body of water, the final sample would preferably be a composite of a number of subsamples taken at various locations and water depths. If samples are collected in the area of a fish kill, a minimum of three samples are collected in the kill area, a control sample well above the suspected source of the pollutant, and one or two samples downstream of the kill area if the pesticide is downstream from the area of dying fish. In the case of a tidewater estuary, some modifications in the sampling pattern may be indicated.

As implied by the name, a grab or dip sample would be a surface water sample generally taken by simply filling the sample container by immersing and allowing the bottle or jar to fill up. For sampling at selected depths, devices such as a Precision sewage water sampler or an Esmarch sampler may be utilized. Both devices consist of a metal outer container with a glass bottle inside as the sample collection vessel.

The Precision sampler in which the interior of the collection bottle has free access to the exterior by means of an open tube can be used to draw a composite depth sample. As soon as the device is immersed, collection of the sample is started. By premeasuring the rate of lowering the device to collect a given amount of water, an approximately uniform amount of water can be collected throughout the entire depth sampled.

The Esmarch sampler may be manually opened and closed by means of a chain attached to the bottle stopper. This permits a sample or subsample to be drawn from any given depth simply by lowering the device with the stopper closed, opening it at the proper sampling depth to permit filling of the collection bottle, then closing the stopper and raising the device to the surface.

IV. SAMPLE CONTAINERS AND STORAGE:

Wide mouth glass jars such as the Mason type are recommended as suitable sample containers when the sample is to be 2 liters or less. If the sample is of greater volume than 2 liters, the one gallon glass bottles in which acetone, hexane or petroleum ether are normally sold provide excellent sample containers. Furthermore, the latter require no special precleaning before use. Other glass containers must be scrupulously cleaned and rinsed with some of the same solvent used for subsequent pesticide extraction. All bottle or jar caps should be Teflon or foil lined to prevent contamination of the sample with trace quantities of impurities which may be present in laminated paper liners or in the composition of the material used for the seal in Mason jar lids.

The size of sample is dictated primarily by the expected residue levels. For example, if the sample is collected from a waterway where pesticide levels are expectedly high (such as agricultural run-off), a sample size of 500 to 1,000 ml may be sufficient. If the sample is drawn in connection with a monitoring program where no especially high residues would be expected, a sample size of 2 liters or more may be indicated. Sample containers should be carefully labeled with the exact site, time, date, and the name of the sampler.

Ideally, analysis of the sample should be conducted within a matter of hours from the time of sampling. However, this is frequently impractical in terms of the distance from sampling site to laboratory, and/or the laboratory workload. Samples being examined solely for organochlorine residues may be held up to a week under refrigeration at 2 to 4°C. Those intended for organophosphorous or carbamate analysis should be frozen immediately after drawing sample and should be extracted no more than 4 days after sampling. These classes of pesticides undergo degradation very rapidly in the aqueous medium.

Every effort should be made to perform the solvent extraction setep at the earliest possible time after sampling, irrespective of the class of pesticides suspected as being present. The resulting extracts may then be held for periods up to three or four weeks at -15 to 20°C before conducting the adsorbent partitioning and determinative portions of the analysis. The reader is referred to Table 1 at the end of Section 10,A. These data show the degradation rate of 29 pesticides in water at ambient temperature in sealed containers (3).

V. OTHER SAMPLING METHODS (Reference (4), Outline 22):

Continuous and automatic samplers of various types are appropriate for sampling flowing rivers and streams. Samplers have been designed to collect water samples at a rate proportional to either water flow or time. Equipment is now available for collecting proportionalized grab samples from gauges and instrumented streams that are proportional to the flow of the stream. This method is particularly useful, in fact required, to determine the total discharge load of a pesticide from a stream. For additional details, see reference (4), outline 22.

Carbon adsorption is a standard method for continuous sampling of water. The technique involves passage of a continuous, constantly controlled volume of water through a column of activated carbon. The major advantages of this method are that it takes a continuous sample and that it yields sufficient quantities of extract for corroborative, qualitative analyses.

The precision of the method appears to be satisfactory, but the quantitative efficiency is open to many questions. Efficiency of adsorption has already been found to vary dramatically, depending on the rate of flow through the column and the total volume passed. A broad spectrum of organics are adsorbed by the carbon, but it has been estimated that perhaps 95% of the total organic load passes through. Many pesticides are adsorbed by activated carbon, but little is known at present about the efficiency of adsorption for specific pesticides. Quantitative statements of pesticide concentration based on carbon adsorption should be restricted to the "it is certain that no less than (X) amount was present," variety.

Besides carbon, many other filter materials have been recommended for continuous samplers, and continuous liquid-liquid extractors are also available.

VI. EQUIPMENT:

1. Gas chromatograph fitted with an electron capture detector and a flame photometric detector with a 526 nm P filter (thermionic detection may be substituted for the FPD). GLC columns, borosilicate glass, 1.8 m x 4 mm i.d., packed with 1.5% OV-17/1.95%

OV-210, and 5% OV-210, both coated on Gas-Chrom Q, 80/100 mesh, operated with specific parameters given under Gas Chromatography, Section IX. Criteria for high sensitivity in the GLC system are set forth in Section 4,A,(4), Page 4 for the EC detection mode, and in Section 4,B,(2), Page 3 for the FPD mode. These should be carefully noted.

2. Chromaflex columns, size 22, 7 mm i.d. x 200 mm, Kontes 420100.

3. Chromaflex column, 22 mm i.d. x 300 mm, size 241, Kontes 420530.

4. Rinco evaporator, rotating, such as Scientific Glass Apparatus Co. E-5500 or E-5500-1, with appropriate stand.

5. Variac or comparable voltage control regulator.

6. Water bath for operation at 35°C.

7. Vacuum source of 125 mm Hg, optimally.

8. Kuderna-Danish evaporators, 250 ml, Kontes 570001.

9. Centrifuge tubes, conical, 15 ml, graduated, Corning No. 8082 with Teflon lined plastic screw caps, thread finish 415-15, Corning 9998.

10. Tubes, culture, screw caps with Teflon liner, 16 x 125 mm, Corning 9826.

11. Evaporative concentrator tubes, 10 ml, graduated from 0.1 to 10.0 ml, size 1025 with outer joint ₮ 19/22, Kontes 570050.

12. Tube heater with aluminum block containing 18 mm (3/4 inch) holes, Kontes 720000 (a water bath can be used as a substitute).

13. Mixer producing a tumbling action at ca 50 rpm (Fisher Roto-Rack or equivalent).

14. Prepurified nitrogen source with 3-stage regulation to produce a gentle stream of gas through an extruded tip of glass or stainless steel.

15. Vortex mini-mixer.

16. Disposable Pasteur pipets, Fisher 13-678-5A or equivalent.

VII. SOLVENTS AND REAGENTS:

1. Methylene chloride, hexane, benzene, acetonitrile, acetone, and methanol, all of pesticide quality.

2. Silica gel, Woelm, activity grade I, activated for 48 hours at 175°C before use. Prepare final deactivated material by adding 1.0 ml of water to 5.0 g silica gel in a vial with a Teflon-lined screw cap. Cap tightly and mix on the Roto-Rack for 2 hours at ca 50 rpm. Discard deactivated silica gel after 5 days.

   NOTE: It is recommended that the amount of silica gel activated at 175°C be restricted to the quantity needed for immediate deactivation.

3. Sodium sulfate, granular, anhydrous. Purify by Soxhlet extracting with methylene chloride for ca 60 discharge cycles.

4. 1-Fluoro-2,4-dinitrobenzene (FDNB), J. T. Baker 5-M478 or equivalent. Prepare a 1% reagent solution in acetone.

5. Sodium borate buffer, 0.1 M solution of $Na_2B_4O_7.10\ H_2O$, pH 9.4, J. T. Baker 3568 or equivalent.

6. Carborundum chips, fine. These should be purified as described for sodium sulfate in Item 3 of this section if a precheck indicates any contamination problems.

7. Glass wool, preextracted with methanol, acetone, and methylene chloride to remove any contaminants.

8. "Keeper" solution, 1% paraffin oil, USP grade, in hexane.

9. Eluting solutions:

   Fraction I - hexane

   Fraction II - benzene-hexane (60:40 v/v)

   Fraction III - acetonitrile-benzene (5:95 v/v)

   Fraction IV - acetone-methylene chloride (25:75 v/v)

10. Contaminant-free water. To 1500 ml of distilled water in a 2 L separatory funnel add 100 ml methylene chloride, stopper, and shake vigorously for 2 minutes. Allow the phases to separate,

discard the solvent layer, and repeat the extraction with another 100-ml portion of methylene chloride. Drain the double-extracted water into a glass stoppered bottle for storage, withdrawing 500 ml to serve as a reagent blank with each set of samples.

11. Pesticide reference standards, analytical grade.

VIII. SAMPLE EXTRACTION AND CONCENTRATION:

1. Transfer 500 ml of water to a 1 L separatory funnel and add 10 g anhydrous sodium sulfate and 50 ml of methylene chloride. Shake vigorously for 2 minutes and allow a sufficient length of time for complete phase separation.

   NOTES:

   1. If the expected pesticide concentration is extremely low, i.e. under .04 µg/L, it may be advisable to increase the initial sample to 1000 to 2000 ml. In this case, the volume of methylene chloride should be increased to 75 ml and the separatory funnel size to 2 or 3 L.

   2. To avoid troublesome caking of the sodium sulfate at the bottom of the funnel, shaking should be conducted instantly after adding the sodium sulfate.

   3. At this point a reagent blank of 500 ml of the preextracted water should be carried through all procedural steps in exactly the same manner as the sample(s).

2. Place a small wad of glass wool at the bottom of a 25 x 300 mm Chromaflex column and add a 2 in. depth of anhydrous sodium sulfate. Position the tip of the column over a Kuderna-Danish assembly consisting of a 250 ml K-D flask attached to a 10 ml evaporative concentrator tube containing two or three carborundum chips and 5 to 10 drops of keeper solution.

3. Drain the lower layer (methylene chloride phase) from the separatory funnel through the sodium sulfate column, taking care to avoid the transfer of any of the aqueous phase.

4. Add 50 ml more of methylene chloride to the aqueous phase in the funnel. Stopper and repeat the 2-minute shaking, phase separation, and draining of the organic layer through the sodium sulfate column into the K-D flask.

   NOTE: It is not uncommon with highly polluted water samples to encounter persistent and sometimes severe emulsion problems at the methylene chloride-water interface. When this occurs, for example, in the extraction of some waste-water samples containing high surfactant concentrations, it is inadvisable to pass the methylene chloride phases through the sodium sulfate because the aqueous emulsion tends to clog the column and make filtration difficult. A good way to cope with an emulsion is to pack a filter tube (A. H. Thomas 4797-N15 or equivalent) with a 25 mm thick pre-washed glass wool pad and pass the extract containing the emulsion through this filter into a 400 ml beaker, applying air pressure if necessary. If the emulsion persists on the second methylene chloride extraction, this treatment is repeated. The glass wool pad is then rinsed with 25 ml of methylene chloride, collecting the extract and washing on the surface of the filtrate, a second glass wool filter is set up and the operation is repeated.

5. Connect the K-D flask to the rotary evaporator and incline the assembly to an angle approximately 20° from the vertical, with the concentrator tube about half immersed in a water bath previously adjusted to 35°C. Turn on the rotator, adjusting speed to a slow spin. Switch off the bath heat and apply vacuum to the evaporator at a pressure of ca 125 mm of Hg.

   NOTE: The recommended adjustments of temperature, vacuum, and the pitch of the assembly should resutl in a steady boiling action and with no bumping. The pitch should be such that no extract condensate collects in the lower position of the K-D flask. (See Figure 1.)

6. Continue evaporation until the extract is condensed to ca 4 ml, remove the assembly from the water bath, and rinse down the walls of the flask with 4 ml of hexane delivered with a disposable pipet.

7. Disconnect the concentrator tube from the K-D flask, rinsing the joint with ca 2 ml of hexane delivered with a disposable pipet.

8. Place the tube under a gentle stream of nitrogen at ambient temperature and concentrate the extract to ca 0.5 ml.

   NOTE: Under no circumstances should air be used for the blow-down as certain organophosphorus and carbamate compounds (and even low concentrations of some organohalogens) may not survive the oxidative effects.

IX. SILICA GEL FRACTIONATION AND CLEANUP:

Before starting the following steps, place 10 drops of the paraffin oil-hexane keeper solution in the two 15 ml centrifuge tubes intended as the receivers for the eluates of Fractions III and IV.

1. Prepare a silica gel column as follows:
    a. Lightly plug a size 22 Chromaflex column with a small wad of preextracted glass wool.
    b. Add 1.0 g of deactivated silica gel, tapping firmly to settle, then top with 1 in. of anhydrous sodium sulfate and again tap firmly.
    c. Pass 10 ml of hexane through the column as a prewash, discarding the eluate.
2. When the last of the prewash hexane just reaches the top surface of the sodium sulfate, quickly place a 15 ml conical centrifuge tube under the column, and using a disposable pipet, carefully transfer the 0.5 ml of sample extract to the column. When this has sunk into the bed, rinse the walls of the centrifuge tube with 1.0 ml of hexane, and, using the same disposable pipet, transfer this washing increment to the column. Repeat this 1.0 ml hexane wash twice more and finally add 6.5 ml hexane to the column. The resulting 10 ml total effluent is Fraction I.

    NOTES:

    1. There must be no interruption of the procedure during this step. Extreme care should be taken to apply the sample to the column at the precise moment the last of the hexane prewash reaches the top surface of the column.
    2. Faultless technique is required in this stip to avoid any losses, particularly during the transfer of the 0.5 ml concentrated extract and the first rinse. All the pesticide derived from the original sample is concentrated in this very miniscule extract. The loss of one drop may introduce a recovery error of at least 10%.
3. Immediately position another 15 ml centrifuge tube under the column and pass through the column 15 ml of the benzene-hexane (60:40 v/v) eluting solution. This is the Fraction II eluate.

4. Make a third elution with 15 ml of the acetonitrile-benzene solution (5:95 v/v). This eluate is Fraction III.

5. A fourth elution fraction is necessary if there is reason to suspect the presence of crufomate, dicrotophos, dimethoate, mevinphos, phosphamidon, or the oxygen analogs of diazinon and malathion. The elution solution is 15 ml of acetone-methylene chloride (25:75 v/v). This is Fraction IV.

6. Place the eluates under a gentle nitrogen stream at ambient temperature and concentrate as follows:

   a. Fractions I and II to ca 3.0 ml, rinse down the tube sidewalls with ca 1.5 ml hexane and adjust the volume to exactly 5.0 ml with hexane. Cap the tubes tightly and mix on the Vortex mixer for one minute.

   b. Fractions III and IV to 0.3 ml, rinse tube sidewalls with hexane, and dilute back to exactly 5.0 ml with hexane.

      NOTE: Fractions III and IV contain eluant solvents which may interfere in the GLC determination, whereas those solvents in Fractions I and II would create no such problems. For this reason, Fractions III and IV are reduced to a lower volume to remove the original solvents.

7. Fractions II and III may contain carbamates as well as organophosphorus compounds. Gas chromatography of organophosphorus compounds by flame photometric detection is conducted on the eluates adjusted to 5.0 ml. When this has been completed, the tubes are placed back under a nitrogen stream, and the eluates are concentrated to 0.1 ml preparatory to derivatization of the carbamates which may be present.

   NOTE: The principal reason for concentrating this eluate to 0.1 ml is to reduce the volume of benzene which could interfere in the subsequent derivatization reaction.

X. CARBAMATE DERIVATIZATION:

1. Add 0.5 ml of the FDNB-acetone reagent solution and 5.0 ml of sodium borate buffer solution to the tubes containing the 0.1 ml of Fractions II and III, and add the same reagents to an empty tube to serve as a reagent blank.

NOTE: At this point, if any specific carbamate is suspected, prepare a solution of known concentration from a primary reference standard. A concentration of 5 µg per ml in acetone may be appropriate. This should be carried through the entire procedure starting with this step in exactly the same manner and at the same time as the unknowns.

2. Cap the tubes tightly and heat at 70°C for one hour in the heating block or in a water bath.

3. Cool the tubes to room temperature and add 5.0 ml hexane to each tube. Shake vigorously for 3 minutes, either manually or on a wrist action mechanical shaker.

4. Allow the layers to separate and carefully transfer 4 ml of the hexane (upper) layer to a vial or test tube which can be stoppered tightly.

XI. GAS CHROMATOGRAPHY:

For multiresidue analysis of samples with unknown pesticidal contamination, two GLC columns yielding divergent compound elution patterns will aid confirmation. Two such columns are 5% OV-210 and 1.5% OV-17/1.95% OV-210. For EC detection, the column oven should be set at 200°C for the mixed column and at 180°C for 5% OV-210 (see exception for carbamates given under XI,5). Carrier gas flow should be set to produce an absolute retention time of 16-19 minutes for p,p'-DDT.

Sensitivity levels for both EC and FPD detectors should be carefully established before starting chromatographic determination. The majority of water samples will contain extremely low pesticide concentrations, and, therefore an insensitive GLC system will severely handicap the analysis. See Sections 4A and 4B of this manual for recommended criteria.

The majority of the halogenated pesticides will be found in Fractions I and II, with a few of the more polar compounds in Fraction III. Most of the organophosphorus compounds will be in Fractions II and III, none in Fraction I, and a very few in Fraction IV. Carbamates are eluted in Fractions II and III (Tables 2-4).

The analyst is referred to Section 4,A(4) of this Manual, pages 2 and 3, 12/2/74 revisions, for a time-saving procedure for tentative peak identification and choice of quantitation standards.

A number of organophosphorus compounds chromatographed with the FPD detector require considerable column preconditioning by repetitive injection of standards of relatively high concentration before attempting quantitation. Failure to carefully monitor linearity of response may result in erroneous quantitative values.

Typical gas chromatograms of silica gel column fractions are shown in Figures 2-4. Figure 3 illustrates the electron capture gas chromatography of chlorinated pesticides, Figure 4 the chromatography of organophosphates with FPD detection, and Figure 2 a chromatogram of dinitrophenyl ether derivatives of carbamates detected by electron capture.

XII. RECOVERY AND DETECTION DATA:

Recovery data for the extraction step alone and the total procedure including silica gel chromatography, and the concentration levels tested are shown in Tables 2-4. Water samples of 500 ml are suitable for detection at these concentration levels. Of the 42 halogenated compounds evaluated, reproducible recoveries of 80% or more were obtained for 31. Gas chromatography linearity problems were encountered with captan and folpet, and a sizable portion of lindane was lost during silica gel fractionation.

Thirty-one of the 38 organophosphorus compounds were recovered in the 80+% range and six between 60 and 79%. Reproducible and satisfactory recoveries were not achieved for carbophenoxon, disulfoton, methamidophos, monocrotophos, and oxydemeton methyl. Of these five compounds, excellent extraction efficiency was observed for carbophenoxon and disulfoton, but complete loss was experienced on the silica gel column. Six compounds were partially recovered in the 0-60% range. Of the 17 OP compounds yielding total recoveries of less than 80%, six of these gave over 90% extraction recovery, but losses occurred during silica gel chromatography.

Final recoveries after fractionation were acceptable for the carbamates metalkamate, carbofuran, methiocarb, and propoxur. Acceptable recoveries were obtained for aminocarb and carbaryl by direct derivatization and gas chromatography of the concentrated methylene chloride extract, by-passing silica gel fractionation which caused losses for these two compounds. Recoveries of mexacarbate were highly inconsistent, both for direct analysis of spiked methylene chloride or water extracts. Silica gel fractionation of this compound resulted in further losses.

XIII. MISCELLANEOUS NOTES:

1. The recommended operation of the concentrator shown in Figure 1 is unusual for pesticide analysis. Customarily, solvent evaporation is achieved by immersing the concentrator tube in a water bath at a higher temperature than the boiling point of the solvent, or the flask is attached to a conventional rotary evaporator. The system shown in Figure 1 achieves two important objectives: the extract is exposed to a maximum temperature of less than 35°C to minimize degradation of heat labile compounds; and the concentrated extract is confined to one container, thereby eliminating need for a transfer. Using the temperature and vacuum levels specified in Section VIII, 100 ml of methylene chloride extract can be reduced to 5 ml in ca 20 minutes in this apparatus.

2. The activity and performance of deactivated silica gel changes in a matter of days. It is desirable to deactivate only the amount required for a 2 or 3 day period. Continuous storage of activated silica gel at 175°C may result in a shift of the compound elution pattern of deactivated columns prepared from this adsorbent. The quantity of silica gel activated should be limited to a one week supply.

3. The derivatization procedure for carbamates is based on work reported by Holden (5) wherein phenols were formed by hydrolysis in a borate buffer followed by reaction with FDNB to form 2,4-dinitrophenyl ethers. This procedure is superior to derivatization with pentafluorophropionic anydride, as used by Sherma and Shafik (2). With the latter method, considerable masking of derivative peaks by EC detection is observed, and, in addition, most of the peaks elute so early and are so poorly resolved that quantitation is difficult. The Holden method of reaction of intact carbamates with FDNB reagent produces peaks which elute significantly later than those resulting from reagent impurities or other contaminants (Figure 4).

4. Recoveries of OP pesticides were found, in general, to be far better when methylene chloride-extracted water rather than unextracted distilled water was used as the spiking substrate to evaluate this procedure. Therefore, unextracted distilled water was used for all recovery studies. As a further test, a sample of water was obtained a few hundred yards downstream from the outfall of a large chemical manufacturing plant and was fortified with a mixture of pesticides and analyzed using the extraction and silica gel fractionation steps. Although a few extraneous peaks were observed with the electron capture

detector, no significant interference with pesticide peaks occurred. This indicates the applicability of the method to real-life water samples.

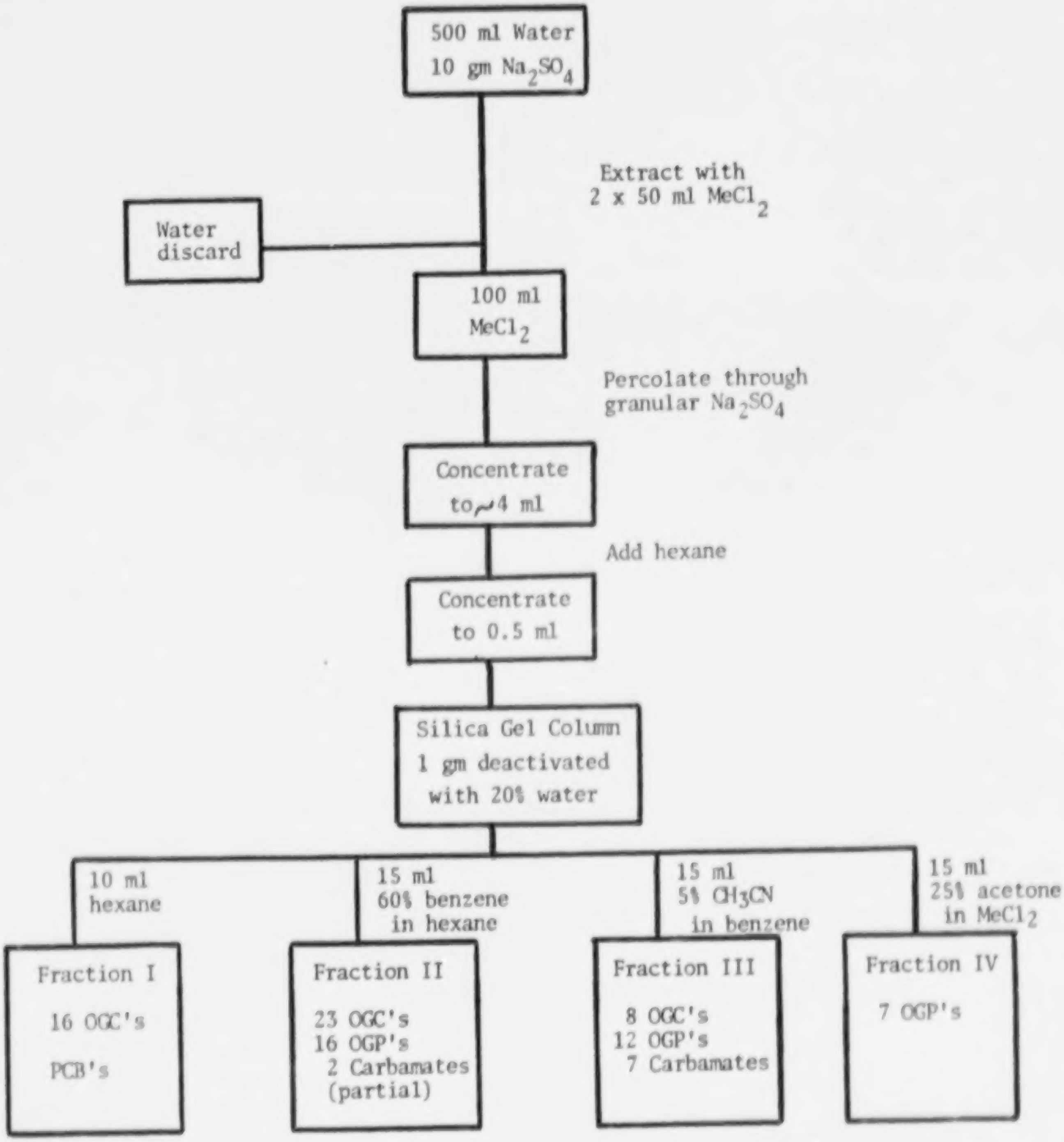
5. The OV-210 GLC column oven can be operated at an elevated temperature (e.g., 215 to 220°C) to expedite elution of the carbamate DNFB derivatives which have high retention times.

XIV. ANALYTICAL QUALITY CONTROL:

1. It is strongly recommended that selected analytical grade standards of known concentrations be analyzed in parallel in each individual sample or set of samples. This will increase confidence in qualitative and quantitative results and will alert the analyst to any shifts in the compound elution pattern from the silica gel column. Certain compounds may elute in different fractions than those shown in Tables 2-4 when different lots of silica gel are used or as atmospheric conditions, particularly relative humidity, vary.

2. Interpretation of chromatograms should be carefully made, based on elutoin patterns from the two dissimilar GLC columns and detectability by the EC and FPD detectors. Further confirmation of compound identity should be made by such techniques as TLC, microcoulometric or Coulson conductivity detector response, p-values, or coupled GLC MS if the latter equipment is available. Confirmatory procedures are discussed in Section 8 of the EPA Quality Control Manual.

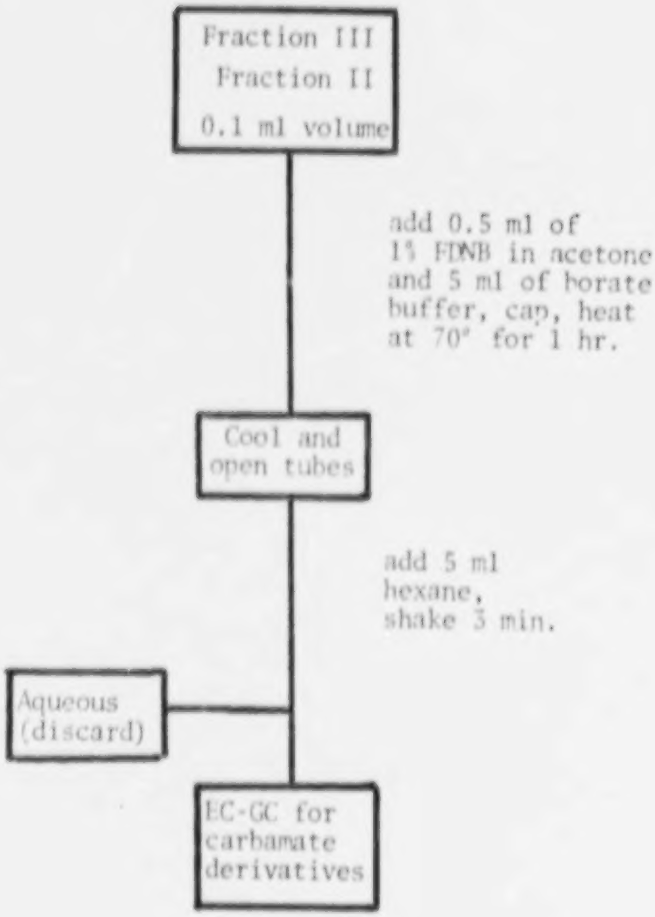
Pesticides in Water -- Thompson et al., 1976


500 ml Water
10 gm Na_2SO_4
Extract with 2 x 50 ml $MeCl_2$
Water discard
100 ml $MeCl_2$
Percolate through granular Na_2SO_4
Concentrate to ~4 ml
Add hexane
Concentrate to 0.5 ml
Silica Gel Column
1 gm deactivated with 20% water
10 ml hexane
15 ml 60% benzene in hexane
15 ml 5% CH_3CN in benzene
15 ml 25% acetone in $MeCl_2$
Fraction I
16 OGC's
PCB's
Fraction II
23 OGC's
16 OGP's
2 Carbamates (partial)
Fraction III
8 OGC's
12 OGP's
7 Carbamates
Fraction IV
7 OGP's

Scheme I

Pesticides in Water -- Thompson et al.

Carbamate Derivatization


Fraction III
Fraction II
0.1 ml volume
add 0.5 ml of 1% FDNB in acetone and 5 ml of borate buffer, cap, heat at 70° for 1 hr.
Cool and open tubes
add 5 ml hexane, shake 3 min.
Aqueous (discard)
EC-GC for carbamate derivatives

410-

Scheme II

Table I. Persistence of Compounds in River Water in Terms of Percentage Recovery

Compound	Original compound found[a], %				
	0-time	1 wk	2 wk	4 wk	8 wk
Organochlorine compounds					
BHC	100	100	100	100	100
Heptachlor	100	25	0	0	0
Aldrin	100	100	80	40	40
Heptachlor epoxide	100	100	100	100	100
Telodrin	100	25	10	0	0
Endosulfan	100	30	5	0	0
Dieldrin	100	100	100	100	100
DDE	100	100	100	100	100
DDT	100	100	100	100	100
DDD	100	100	100	100	100
Chlordane (tech.)	100	90	85	85	85
Endrin	100	100	100	100	100
Organophosphorus compounds					
Parathion	100	50	30	<5	0
Methyl parathion	80	25	10	0	0
Malathion	100	25	10	0	0
Ethion	100	90	75	50	50
Trithion	90	25	10	0	0
Fenthion	100	50	10	0	0
Dimethoate	100	100	85	75	50
Merphos	0	0	0	0	0
Merphos recov. as Def	100	50	30	10	<5
Azodrin	100	100	100	100	100
Carbamate compounds					
Sevin	90	5	0	0	0
Zectran	100	15	0	0	0
Matacil	100	60	10	0	0
Mesurol	90	0	0	0	0
Baygon	100	50	30	10	5
Monuron	80	40	30	20	0
Fenuron	80	60	20	0	0

[a] Pesticide concentrations were 10 μg/liter. Recoveries were rounded off to the nearest 5%.

TABLE 2. RECOVERIES OF 42 ORGANOCHLORINE COMPOUNDS

		Recoveries in Percent					
			Silica Gel Partitioning				
Compound	Conc. (ppb)	Extraction Only	(Elution Fraction) I	II	III	IV	Total
Aldrin	.20	89	88				88
Atrazine	66.5	101			99		99
α-BHC	.09	91	76	4			80
β-BHC	.47	99		88			88
γ-BHC (Lindane)	.12	90	16	51			67
Captan[a]	6.50	100+			100+		100+
CDEC	.27	63		41			41
Chlorbenside	.47	91	62				62
Chlordane	1.54	85	89	1			90
Chlordecone (Kepone)	3.64	72		18	8		26
2,4-D, butyl ester	4.08	93			90		90
2,4-D, butoxyethanol ether ester	8.65	109		9	90		99
2,4,-D, isooctyl ester	3.28	105		95			95
2,4-D, isopropyl ester	3.38	75		71			71
DCPA	.50	98		84			84
p,p'-DDD	.80	97	94				94
p,p'-DDE	.45	96	101				101
o,p'-DDT	1.05	94	93				93
p,p'-DDT	1.58	104	98				98
Dichlone	13.4	85		79			79
Dieldrin	.72	97					96
Dilan	2.42	97		94			94
Dyrene	8.70	95		77			77

---

[a] Non-linear response

(Continued)

TABLE 2. (CONTINUED)

Compound	Conc. (ppb)	Extraction Only	Recoveries in Percent Silica Gel Partitioning (Elution Fraction) I	II	III	IV	Total
Endrin	1.11	105		98			98
Endosulfan (Thiodan)	.53	91	24	80			104
Folpet[b]	1.5	100		131			131
Heptachlor	.18	90	79				79
Heptachlor Epoxide	.31	91		89			89
Hexachlorobenzene (HCB)	.20	74	96				96
1-Hydroxychlordene	.34	81			82		82
Methoxychlor	5.70	97		104			104
Mirex	2.35	83	83				83
PCNB	.10	87	88				88
Perthane	66.5	89	80	15			95
Simazine	66.5	71			28		28
2,4,5-T, butyl ester	2.00	102		99			99
2,4,5-T, butoxyethanol ether ester	3.00	103		71	23		94
2,4,5-T, isooctyl ester	6.05	109		97			97
Tetradifon (Tedion)	2.99	103		102			102
Toxaphene	22.3	103	93				93
Aroclor 1254	25.6	93	96				96
Aroclor 1260	25.6	93	92				92

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[b] Non-linear response

TABLE 3. RECOVERIES OF 38 ORGANOPHOSPHOROUS COMPOUNDS

Compound	Conc. (ppb)	Extraction Only	Recoveries in Percent				
			Silica Gel Partitioning				
			(Elution Fraction)				
			I	II	III	IV	Total
Azinphos Methyl (Guthion)	320	78			88		88
Carbophenothion (Trithion)	48	99		93			93
Carbophenoxon	80	94					0
Chlorpyrifos (Dursban)	4	99		87			87
Crufomate (Ruelene)	90	80				58	58
DEF	24	102			90		90
Diazinon	20	108			104		104
Diazoxon	10	92				72	72
Dichlofenthion (VC-13)	1.6	102		102			102
Dicrotophos (Bidrin)	120	17				15	15
Dimethoate (Cygon)	24	40				60	60
Dioxathion (Delnav)	28	103		72	17		89
Disulfoton (Di-Syston)	2.6	92					0
EPN	60	99		96			96
Ethion	20	100		94			94
Ethoprop (Prophos)	2	97			96		96
Fenitrothion (Sumithion)	12	99		84			84
Fenthion (Baytex)	12	93		76			76
Fonofos (Dyfonate)	20	98		78			78
Leptophos (Phosvel)	200	107		91			91
Malaoxon	80	104				50	50
Malathion	4	100			78		78
Methamidophos (Monitor)	200	5					0
Mevinphos (Phosdrin)	6	69			32	33	65

(Continued)

TABLE 3. (CONTINUED)

			Recoveries in Percent				
			Silica Gel Partitioning (Elution Fraction)				
Compound	Conc. (ppb)	Extraction Only	I	II	III	IV	Total
Monocrotophos (Azodrin)	72	0					0
Naled (Dibrom)	56	92			45		45
Oxydemeton Methyl (Metasystox R)	300	67					0
Paraoxon ethyl	40	99			90		90
Paraoxon methyl	36	98			93		93
Parathion ethyl	16	101		99			99
Parathion methyl	16	99		93			93
Phencapthon	60	99		98			98
Phorate (Thimet)	1.3	98		56			56
Phosalone (Zolone)	400	102		91			91
Phosmet (Imidan)	220	82			85		85
Phosphamidon (Dimecron)	80	43				43	43
Ronnel	4	100		96			96
Ronnoxon	120	94			92		92

TABLE 4. RECOVERIES OF 7 CARBAMATE COMPOUNDS

Compound	Conc. (ppb)	Spkd. $MeCl_2$ (No Extract)	Recoveries in percent Silica Gel Partitioning (Elution Fraction) I	II	III	IV	Total
Aminocarb	10	90			59		59
Bux	10	101			93		93
Carbaryl	10	64			68		68
Carbofuran	10	95		4	94		98
Methiocarb	10	94		55	57		112
Propoxur	10	100			99		99
Zectran	10	69			58		58

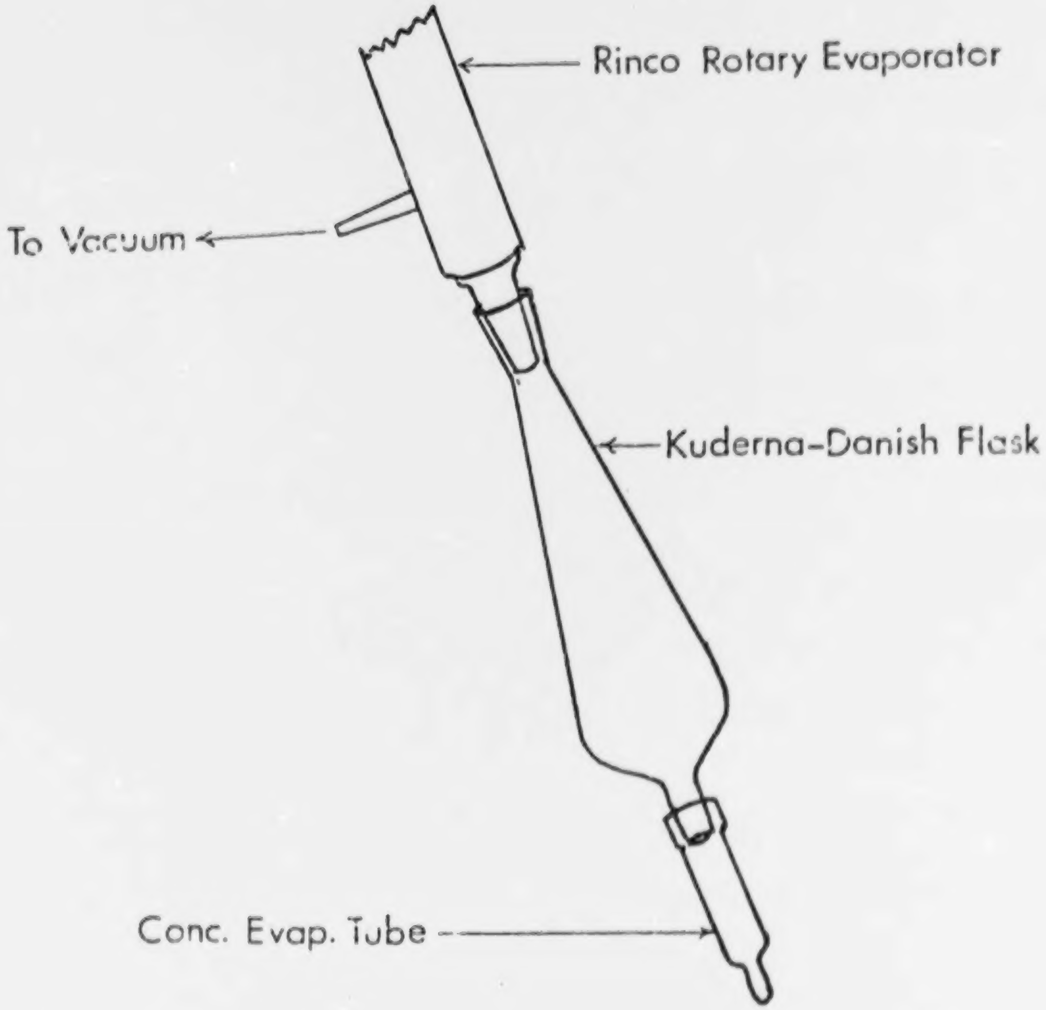
Rinco Rotary Evaporator
To Vacuum
Kuderna-Danish Flask
Conc. Evap. Tube

Fig. 1. Evaporation assembly

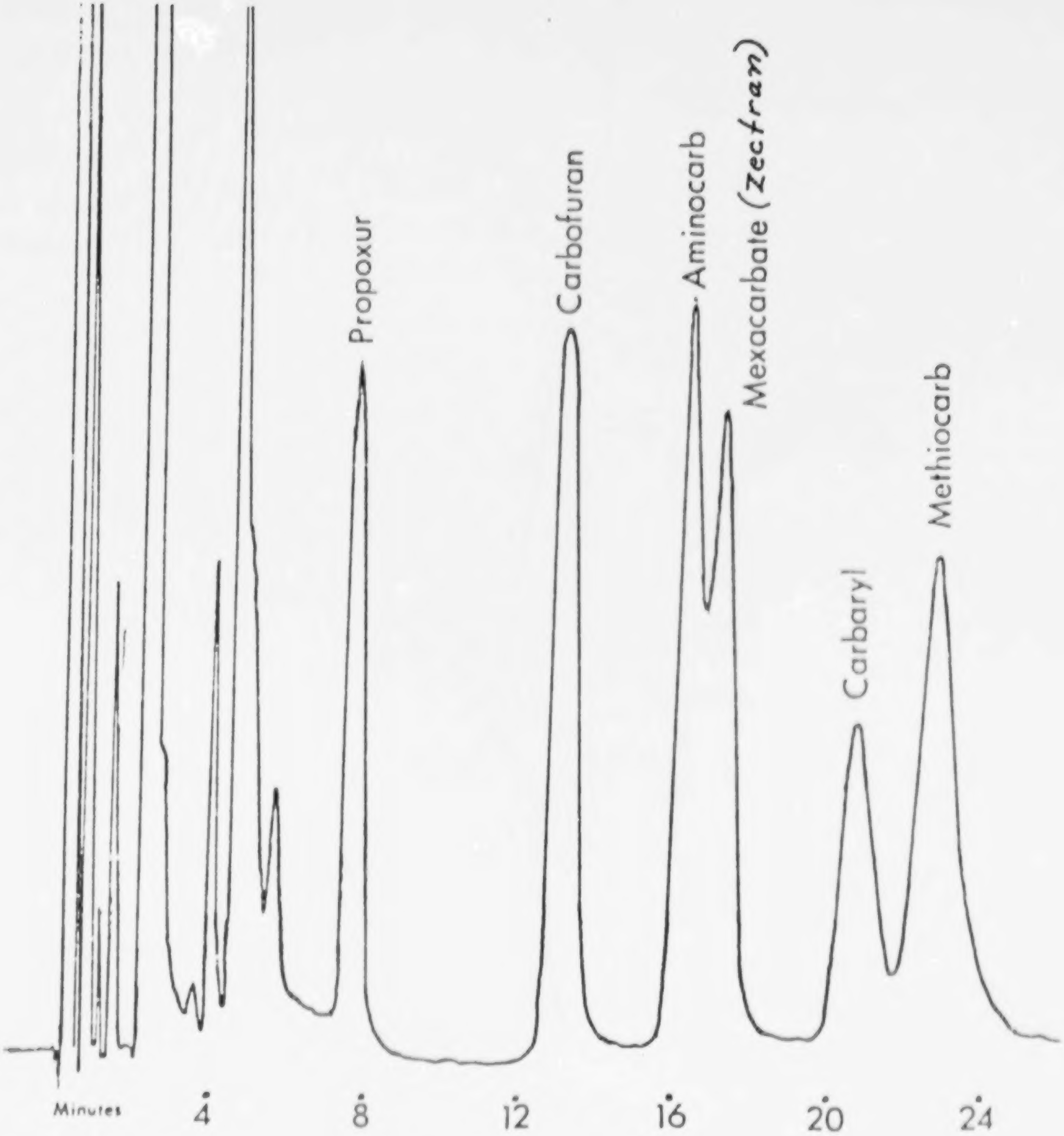

Fig. 2. Six carbamates eluted in Fraction III (portion of carbofuran and methiocarb in Fraction II). 5.0 nanograms of each compound. GC column 5% OV-210.

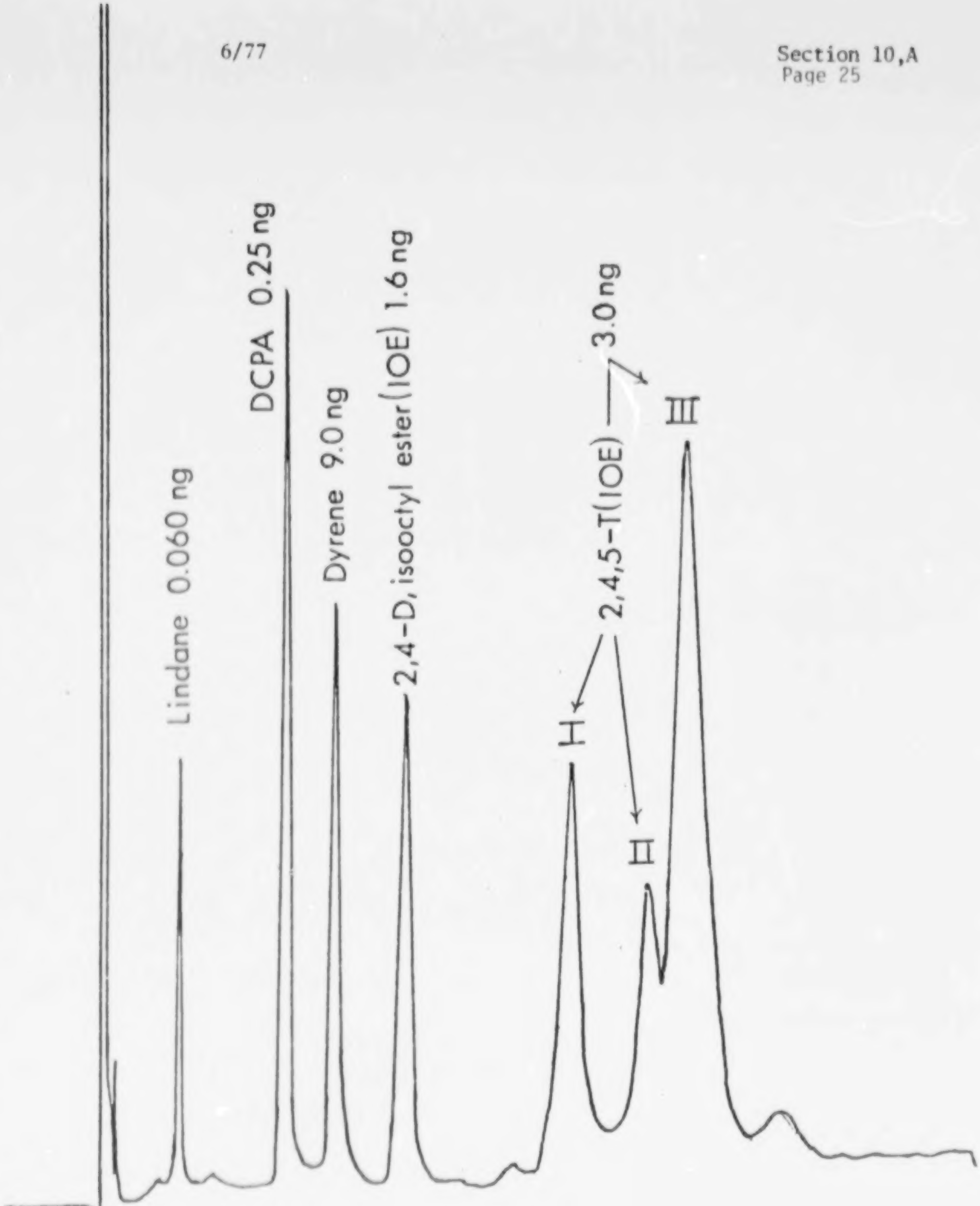

Fig. 3. Five chlorinated compounds eluted in Fraction II. GC column 1.5% OV-17/1.95% OV-210

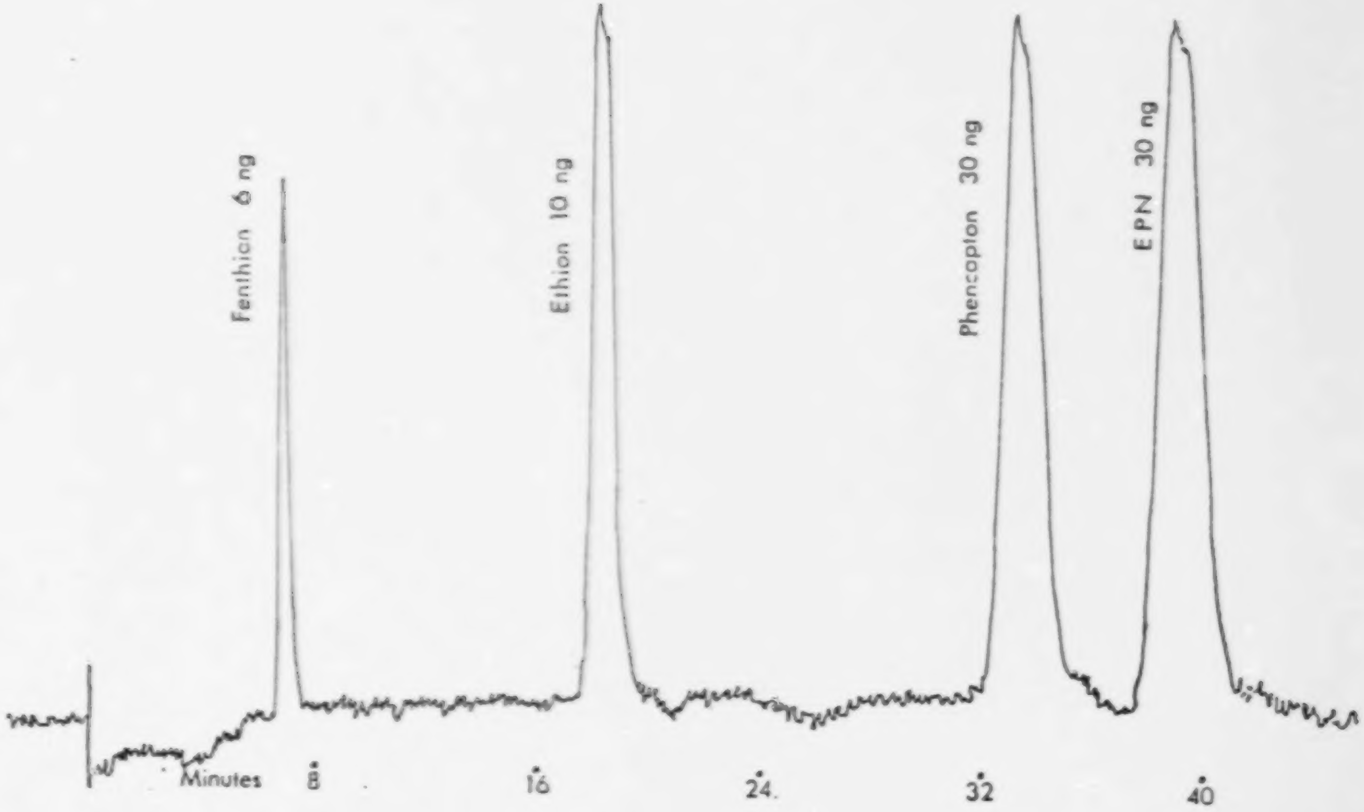

Fig. 4. Four organophosphorous compounds eluted in Fraction II. GC column 1.5% OV-17/1.95% OV-210

DETERMINATION OF SOME FREE ACID HERBICIDES IN WATER

I. INTRODUCTION:

With the intensive use of herbicides for agricultural noxious weed control and direct application in certain waterways for aquatic broadleaf plant control, the environmental chemist is being called upon with increasing frequency to monitor for herbicide residue levels in water. The free acid herbicides, particularly the chlorophenoxys, comprise a commercially important group. The electron capture gas chromatography of these compounds requires that the free acids be converted to derivatives such as the ethyl or methyl esters. One common esterification approach has been through reaction with diazo compounds, but analysts are objecting to the use of these compounds because of their carcinogenicity and other personal safety risks. These extreme hazards dictate the need for very stringent safety precautions in the handling of these reagents. The method described below requires only the normal safety practices customarily used for the handling of compressed gasses, moderately toxic or corrosive materials, and flammable solvents.

REFERENCES:

1. An Improved Gas Chromatographic Method for the Analysis of 2,4-D Free Acid in Soil, Woodham, D. W., Mitchell, W. G., Loftis, C. D., and Collier, C. W.: J. Agr. Food Chem., 19 (1), 186 (1971)

2. A Multiclass, Multiresidue Analytical Method for Pesticides in Water, Thompson, J. F., Reid, S. J., and Kantor, E. J.: Arch. Environ. Contam. Toxicol. in-press (1977), and this Manual, Section 10,A

II. PRINCIPLE:

The pH of the water sample is reduced to 3.0 with H_2SO_4, and extraction and solvent evaporation are carried out as described in Section 10,A for multiresidues in water, utilizing the same roto-vap equipment and taking to dryness under a nitrogen stream. An esterification reagent of 10% BCl_3 in 2-chloroethanol is added, and a prescribed reaction time and temperature are applied. Hexane and Na_2SO_4 solution are added, and, after shaking and phase separation, gas chromatography (EC) is conducted on a concentrate of the hexane layer containing the ethanolic esters. If cleanup is required, the partially deactivated silica gel method described in Section 10,A is used, with all esterified compounds being eluted in Fraction II.

III. EQUIPMENT AND REAGENTS:

1. All equipment and glassware specified for water method in Section 10,A.

2. GLC columns: 5% OV-210, 1.8 m (6') x 4 mm (5/32") i.e. and 1.5% OV-17/1.95% OV-210, same dimensions, both operated at 200°C.

3. Impinger air sampling (bottle only), height 165 mm, outside diam. 41 mm, capacity 125 ml, bottle opening ₮ 24/25, Ace Glass, Inc. #7540.

4. Glass stoppers, 24/25, for bottles in Item 3 above.

5. Magnetic stirring rods, Teflon-coated.

6. Evaporative concentrator tubes, graduated, 25 ml.

7. Disposable pipets.

8. Water bath, 90°C, and ice bath, 0°C.

9. Balance, top loading 1 Kg capacity, accurate to ±0.2 g.

10. Sulfuric acid, conc., reagent grade.

11. Boron trichloride, 1 lb lecture bottle with suitable shut-off valve, Matheson Gas Products.

12. 2-Chloroethanol, 99%, Aldrich Chemical #18,574-4.

    NOTE: Should be redistilled in a hood just prior to use.

13. Sodium sulfate, anhydrous, reagent grade, preextracted with $MeCl_2$. Prepare a 7% solution in water.

14. pH Indicator paper, range 0-3.0.

15. Reference standards of analytical grade purity.

16. Preparation of 10% BCl_3 in 2-chloroethanol Esterification Reagent:

    a. Place a magnetic stirring rod on the bottom of a 125 ml impinger cylinder (Item 3) and position cylinder on top loading balance. Load beams to the tare weight of the cylinder and rod and then increase the beam weight by 90.0 grams.

b. Add 2-chloroethanol to the cylinder until the beam indicator shows a 90-gram addition. Record this weight.

c. Place the cylinder in an ice bath and allow time for temperature equilibration. Insert a glass gas delivery tube into the cylinder, extending to ca 5 mm from the bottom of the cylinder. Extend the glass tubing line from the cylinder to the BCl_3 lecture bottle through a glass trap to prevent any back suction of solution from the cylinder to the gas bottle.

d. Place the cylinder on the magnetic stirrer plate and increase the stirring base velocity to full speed. Open the gas bottle valve to bubble BCl_3 into the chloroethanol at a vigorous rate so that no bubbles of BCl_3 escape unabsorbed from the chloroethanol. Observe the rise in volume of the solution in the cylinder. When it appears that the level of the solution may be approaching a 10 ml addition, shut off the gas flow, remove the cylinder from the bath and place on the balance for check weighing. Continue gas addition as necessary for the final weight to show a 10 g addition of BCl_3.

NOTES:

1. All reagent preparation steps should be conducted in an exhaust hood.

2. It may be found convenient the first time the reagent is prepared to scratch marking lines on the cylinder with a diamond pencil at the liquid level of the pure chloroethanol after chilling and also at the level reached by the addition of 10 g of BCl_3. These will serve as reference points for the preparation of future lots of the reagent.

3. The air sampling impinger cylinder (Item 3), although intended for an entirely different use, was found ideal in type, size, and shape for this application.

4. The reagent, if kept stoppered and refrigerated, was found to be stable up to 30 days. Although no observations were made after this length of time, it may well be stable for longer periods.

IV. EXTRACTION:

The sample size should be gauged by the expected concentration levels of herbicide residues in the sample. If fairly high levels are expected, such as in a direct run-off area from a spray application, a 500 ml sample may be appropriate. A liter, however, may be indicated for lower concentrations, depending, in part, on the specific compounds. MCPB, for example, is somewhat unresponsive and will require a larger sample for detection; on the other hand, silvex is highly responsive and, therefore, far less is required in the final extract for chromatography.

1. Add conc. H_2SO_4, drop by drop, to the sample until a pH of 3.0 or slightly less is observed by testing with pH indicator paper of a 0-3.0 pH range. Generally 4-6 drops will suffice.
2. From this point onward, follow all steps and details under SAMPLE EXTRACTION AND CONCENTRATION, Section 10,A,VIII, finishing at Step 6 with a $MeCl_2$ concentrate of ca 4.0 ml in a 10 ml concentrator tube.
3. With a disposable pipet, transfer the contents of the 10 ml concentrator tube to a 25 ml graduated concentrator tube, rinse down the sides of the 10 ml tube three times with 0.5 ml of hexane and transfer each wash to the larger tube.
4. Place the 25 ml tube under a gentle stream of nitrogen at room temperature and evaporate _just_ to dryness.

V. ESTERIFICATION:

1. Add 1.0 ml of the esterification reagent to the 25 ml concentrator tube allowing the reagent to flow down the entire inner walls of the tube.
2. Immerse tube in a 90°C water bath for 10 minutes.
3. Remove the tube from the bath, cool to room temperature, and add 5.0 ml of hexane and 10 ml of 7% Na_2SO_4 solution. Stopper the tube and mix at high speed on a Vortex mixer.
4. Allow the layers to separate and proceed with GLC on the hexane phase.

VI. GAS CHROMATOGRAPHY:

Use a column consisting of 5% OV-210 coated on Gas Chrom-Q, 80/100 mesh. At the oven temperature parameters given in Table 1, approximately the same RRT$_A$ values should be expected from columns of 5% QF-1 or 5% SP-2401.

The confirmation column of 1.5% OV-17/1.95 OV-210 finds its counterpart in RRT$_A$ characteristics in a commercially available column of 1.5% SP-2250/1.95% SP-2401.

If the analysis is run on a water sample of completely unknown constituents, the analyst may obtain a clue of the constituent(s) by calculating the RRT$_A$ of any peaks in the sample chromatogram and comparing the calculated RRT$_A$ values with those given in Table 1. However, any reagent blank peaks of comparable RRT$_A$ values and of significant response must be carefully considered.

Further validation should be carried out on an alternate column of entirely different compound elution characteristics.

VII. SILICA GEL FRACTIONATION:

This step is not recommended until gas chromatography is carried out on esterified extract. If the contaminant background is minimal, there is no need to do this procedure. If a significant background is evident and the peaks are obviously of the same approximate RRT$_A$ values as herbicide ester peaks, the fractionation step is necessary.

The fractionating procedure is conducted in the same manner described in Section 10,A,IX, SILICA GEL FRACTIONATION AND CLEANUP, transferring an aliquot of 4.0 ml of the esterfied hexane extract to a 10 ml concentrator tube and reducing the extract volume to 0.5 ml under a nitrogen stream at room temperature. All esterified compounds should elute in Fraction II.

NOTE: The analyst must not overlook the fact that the final sample size will be only 4/5 (0.8) of the original sample due to the 4 ml aliquot taken from the 5.0 ml concentrate.

VIII. QUALITY CONTROL:

If the identity of the free acid herbicide in the sample is known, an SPRM (spiked reference material) of water should be prepared with a compound concentration estimated to be comparable to that of the sample. This SPRM should be carried through the entire procedure in exactly the same manner as the sample. If the recovery from the SPRM should turn out significantly poorer than the recovery shown in Table 1 for the compound, the analyst would be well advised to repeat the work since, in all probability, the validity of the results from the unknown(s) will be no better than that of the SPRM.

TABLE 1. RECOVERY AND RRT_A DATA FOR 11 FREE ACID HERBICIDES. COLUMN 5% OV-210 OPERATED AT 200°C AND CARRIER FLOW OF 60 ML/MIN., ^{63}Ni DETECTOR AT 300°C.

Compound	Conc. in Water (ppb)	RRT_A	Recovery After Extract. and Esterif. (%)	Recovery After Silica Gel Fraction (%)
MCPP	5.0	1.14	109	88
MCPA	3.0	1.35	98	85
Dichlorprop	0.4	1.47	92	83
Fenac	1.36	1.75*	94	86
Naphthalene Acetic Acid	40.0	1.90	97	87
Silvex	0.25	2.15	105	85
2,4-D	4.0	2.19*	90	75
MCPB	68.0	2.66	113	93
2,4,5-T	4.0	3.44*	88	63
2,4-DB	2.0	3.47	90	89
4-(2,4,5-TB)	1.0	5.57	95	91

*These RRT_A values were determined at a column temperature of 180°C.

ORGANOCHLORINE INSECTICIDES IN SOILS AND HOUSEDUST

I. INTRODUCTION:

The analytical method described below is similar in principle to the method presented in the Analytical Manual distributed at the annual Chemist's Meeting in Tucson in 1968. The main difference lies in the incorporation of the standard Mills, Onley, Gaither Florisil cleanup technique for which all laboratories have equipment and a degree of expertise in manipulation. This is preceded by percolation through an alumina column for further removal of contaminants.

II. PRINCIPLES:

Organochlorine pesticides, together with other lipid-soluble substances, are extracted from homogenized samples by continuous Soxhlet extraction with acetone-hexane. Bulk of solvent is removed by evaporation in Kuderna-Danish equipment. Interfering lipid-soluble materials are then partially removed from the extracts by successive cleanup on aluminum oxide and Florisil columns. Extracts are adjusted to appropriate concentration for determinative analysis by EC and FPD confirming as needed by MC and/or TLC.

III. EQUIPMENT:

1. Soxhlet extraction apparatus, complete with 125-ml \$ 24/40 flask, extraction tube with \$ 24/40 lower and \$ 34/45 upper joints and Friedrichs condenser with \$ 34/45 joint. Kimble #24010 or the equivalent for the entire assembly.

2. Soxhlet extraction thimbles, paper, Whatman, 25 x 80 mm, Fisher #9-656-c or the equivalent.

3. Sieves, U.S. Standard, #10 mesh, #18 mesh and #60 mesh with top covers and bottom pans, 8 in. dia. x 2 in. depth, stainless steel.

4. Chromatographic columns, 22 x 300 mm with Teflon stopcock, without glass frit. Size #241, Kontes #420530 or the equivalent.

5. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:

    a. Flask, 250 and 500 ml, stock #K-570001.

    b. Snyder column, 3 ball, stock #K-503000.

   c. Steel springs, 1/2 in., stock #K-662750.

   d. Concentrator tubes, 10 ml grad., size 1025, stock #K-570050.

6. Modified micro-Snyder columns, 19/22, Kontes K-569251.

7. Glass beads, 3 mm plain, Fisher #11 - 312 or equivalent.

8. Evap. concentrator tubes, grad., 25 ml, ₮ 19/22, Kontes #570050.

9. Water or steam bath.

10. Glas Col heating mantles with variable autotransformers, size to match 125-ml Soxhlet flasks.

11. Filter paper, Whatman No. 1, 15 cm.

IV. REAGENTS AND SOLVENTS:

1. Acetone, pesticide quality.

2. Hexane, pesticide quality.

   NOTE: Both solvents must be carefully checked for background contaminants as outlined in Section 3,C of this manual.

3. Extraction mixture - acetone/hexane, 1:1.

4. Aluminum oxide, Merck reagent grade, stock #71695 acid-washed. Prepare for use by shaking with 10% distilled water (w/w) for partial deactivation. Shelf life of 10 days if stoppered tight.

   NOTE: The distilled water must be prechecked for contaminant background. If any interferences are detected, the water must be hexane extracted before use.

5. Diethyl ether - AR grade, peroxide free. The ether must contain 2% (v/v) absolute ethanol. Most of the AR grade ethyl ether contains 2% ethanol, added as a stabilizer, and it is therefore unnecessary to add ethanol unless peroxides are found and removed.

   NOTE: To determine the absence of peroxides in the ether, add 1 ml of ether in a clean 25 ml cylinder previously rinsed with ether. Shake and let stand 1 minute. A yellow color in either layer indicates the presence of peroxides which must be removed before using. See Misc. Note 4 at end of procedure. The peroxide test should be repeated at weekly intervals on any single bottle or can as it is possible for peroxides to form from repeated opening of the container.

6. Eluting mixture, 6% (6+94)-purified diethyl ether - 60 ml is diluted to 1000 ml with redistilled petroleum ether, and anhydrous sodium sulfate (10-25 g) is added to remove moisture.

7. Eluting mixture, 15% (15+85)-purified diethyl ether - 150 ml is diluted to 1000 ml with redistilled petroleum ether, and dried as described above.

   NOTE: Neither of the eluting mixtures should be held longer than 24 hours after mixing.

8. Florisil, 60/100 mesh, PR grade, to be stored at 130°C until used. Furnished by Perrine on order.

   NOTES:

   1. In a high humidity room, the column may pick up enough moisture during packing to influence the elution pattern. To ensure uniformity of the Florisil fractionation, it is recommended to those laboratories with sufficiently large drying ovens that the columns be packed ahead of time and held (at least overnight) at 130°C until used.

   2. Florisil furnished by the Perrine Laboratory has been activated by the manufacturer, and elution pattern data is included with each shipment. However, each laboratory should determine their own pesticide recovery and elution pattern on each new lot received, as environmental conditions in the various laboratories may differ somewhat from that in Perrine. Each new batch should be tested with a mixture of β-BHC, aldrin, heptachlor epoxide, dieldrin, p,p'-DDE, p,p'-DDD, and p,p'-DDT, eluting the standard mixture as described in Section 5,A,(1) of this manual. Dieldrin should elute entirely in the 15% diethyl ether fraction, whereas all other compounds should be in the 6% fraction.

9. Anhydrous sodium sulfate, reagent grade granular, Mallinckrodt Stock #8024 or the equivalent.

   NOTE: When each new bottle is opened, it should be tested for contaminants that will produce peaks by Electron Capture Gas Liquid Chromatography. This may be done by transferring ca 10 grams to a 125 ml Erlenmeyer flask, adding 50 ml pet. ether, stoppering and shaking vigorously for 1 minute. Decant extract into a 100 ml beaker and evaporate down to ca 5 ml. Inject 5 µl into the Gas Liquid Chromatograph and observe chromatogram for contaminants. When impurities are found, it is necessary to remove them by extraction. This may be done using hexane in a continuously cycling Soxhlet extraction apparatus or by several successive rinses with hexane in a beaker. The material is then dried in an oven and kept in a glass-stoppered container.

V. SAMPLE PREPARATION:

1. Soils and vacuum clean bag dusts are analyzed in the air-dry state. If a soil sample is obviously damp, it is allowed to equilibrate its moisture content with room air before handling. Trials have shown that house dust screenings generally contain approximately 0.1% moisture, possibly more in areas of high relative humidity.

2. Vacuum cleaner bag contents are sieved on U.S. Standard #10 and #60 sieves to remove hair, fibers and large particles. The resulting "fines" are separated into sealed glass jars until analyzed. Soils are sifted on a U.S. Standard #18 sieve to remove stones and other foreign material. Store the sieved soil in a sealed glass jar until analyzed.

3. The 15 cm filter paper and the Soxhlet extraction thimbles should be preextracted with the acetone/hexane extraction solvent prior to use. This may be conveniently done by folding several sheets of filter paper and placing in the Soxhlet extractor. Allow to cycle ca 2 hours, remove and dry. Wrap in aluminum foil and store in desiccator. The thimble is similarly preextracted and may be used repeatedly with no need for reextraction as long as it remains in good physical shape.

VI. EXTRACTION:

1. Weigh sample (2 grams of soil or 1 gram of dust) onto a sheet of 15 cm filter paper. Carefully fold paper to form a half-circle with the sample in the center (along the diameter line). Fold in the ends of the half-circle towards the center, the total resulting length to be ca 70 mm; then, starting at the diameter line, roll into an approximately cylindrical shape and insert into the extraction thimble.

2. As a recovery check, another portion of the same dust (or soil) should be spiked and carried through the entire procedure. This is done as follows:

    a. Weigh exactly 3.0 grams of the soil or 2.0 grams of dust into an evaporating dish. Add sufficient hexane to make a slurry.

b. Prepare a standard mixture of the following compounds, the concentration expressed in micrograms per milliliter:*

Lindane----------5.0
Hept. Epoxide----5.0
Aldrin-----------5.0
p,p'-DDE---------7.5
Dieldrin---------7.5
p,p'-DDD--------10.0
o,p'-DDT--------10.0
p,p'-DDT--------10.0

*In case your testing program indicates the presence of any other compounds or metabolites, standards of these should be included.

c. Add 1.5 ml of this mixture to the soil sample or 1.0 ml to dust. Mix gently with a glass rod and evaporate the solvent at 40°C under a nitrogen stream, stirring from time to time.

d. After removal of the solvent, allow the spiked sample to equilibrate to room temperature and humidity, and weigh the sample for extraction as outlined above in Step 1.

3. At this point, a reagent blank should be initiated, starting with the folded filter paper and carrying through the entire extraction, cleanup, and determinative procedures.

4. Place the sample, reagent blank and spiked sample thimbles into separate Soxhlet extractors. Fill the boiling flasks, each containing six glass beads, about half full with the 1:1 acetone/hexane co-solvent, assemble the extraction apparatus, position in the heating mantles, and start extraction

NOTE: Each laboratory will need to determine the setting of their voltage controller. There should be sufficient heat to result in 1 discharge cycle about every 5 minutes, or ca 60 syphon discharges in a 5-hour period. This should be an adequate number of cycles to ensure complete extraction.

5. At the completion of the extraction period disassemble the extraction apparatus, rinsing the joint between flask and extractor with a few ml of hexane.

6. Assemble a Kuderna-Danish evaporator with the 250 ml K-D flask attached to a 10 ml evap. concentrator tube containing one 3 mm glass bead.

7. Transfer the extract from the 125 ml Soxhlet flask to the K-D flask, rinsing the Soxhlet flask with 3 portions of 5 ml each of hexane. Attach the Snyder column and immerce evap.

concentrator tube about 1-1/2 inches into the boiling water bath. Evaporate extract down to ca 3 ml, remove from bath and cool. Extract is now ready for cleanup.

VII. ALUMINA AND FLORISIL PARTITIONING:

1. Prepare an alumina column as follows:

   a. Place a small wad of prerinsed glass wool at the bottom of a 22 x 300 mm chromatographic column.

   b. Add preextracted anhydrous Na_2SO_4 to a depth of 1/2 inch.

   c. Close stopcock and fill column with hexane.

   d. In a 50 ml grad. beaker, fill exactly to the 30 ml mark with alumina (this should be ca 30 grams). Add this slowly to the column, allowing all the alumina to settle to the bottom. Top this with a 1 in. layer of Na_2SO_4. When settling is complete, open stopcock, and allow the hexane to elute through the column down to a point ca 1/8 inch above the top of the upper Na_2SO_4 layer, then close stopcock.

   NOTE: This column packing technique minimizes the density that may be obtained in dry packing. The volume of hexane specified provides sufficient column prerinse.

2. Position a second K-D flask fitted with 10 ml evap. concentrator tube under column.

3. Transfer the 3 ml of concentrated extract from the first K-D evaporation to the column. Rinse tube with three portions of 3 ml each of hexane transferring the rinsings to the column.

4. Open stopcock and add 85 ml of hexane to the column, open stopcock wide and elute into the K-D flask.

5. Concentration of the eluate from the alumina column is conducted exactly the same as outlined above in Step 7 under Sample Extraction, taking extract down to 3 ml. This extract is now ready for Florisil partitioning.

6. Florisil column: Prepare the column as described in Section 5,A, (1) of this manual under FLORISIL FRACTIONATION, Steps 1 and 2, substituting hexane for pet. ether.

7. Assemble two more K-D apparatus but with 500 ml flasks and position the flask of one assembly under the Florisil column.

However, at this point use 25 ml grad. evap. concentrator tubes instead of the 10 ml size for previous concentrations.

8. Using a 5 ml Mohr or a long disposable pipet, immediately transfer the extract from the evaporator tube in Step 5, above, onto the column and permit it to percolate through. Rinse tube with two successive 5 ml portions of hexane, carefully transferring each portion to the column with the pipet.

   NOTE: Use of the Mohr or disposable pipet to deliver the extract directly onto the column precludes the need to rinse down sides of the column.

9. Commence elution with 200 ml of 6% diethyl ether in pet. ether (Fraction I). The elution rate should be ca 5 ml per minute. When the last of the eluting solvent reaches a point ca 1/8 inch from the top of the Na_2SO_4 layer, place the second 500 ml Kuderna-Danish assembly under the column and continue elution with 200 ml of 15% diethyl ether in pet. ether (Fraction II). Place both Kuderna-Danish evaporator assemblies in a water bath and concentrate extract to ca 20 ml.

   NOTE: If there is reason to suspect the presence of malathion in the sample, have a third 500 ml K-D assembly ready. At the end of the 15% fraction elution, add 200 ml of 50% diethyl ether in pet. ether (Fraction III), evaporating the eluate in the same manner.

10. Remove K-D assemblies from bath, cool and rinse § joint between tube and flask with a little pet. ether. Finally, dilute both extracts to exactly 25 ml and proceed with the GLC determinative step.

    NOTE: A relatively high dilution is suggested as it has been observed that reisdues are generally sufficiently high to warrant this. Furthermore, the concentration of contaminants remaining after cleanup is hereby reduced.

VIII. GAS CHROMATOGRAPHY:

1. Inject 5 µl of each fraction extract into the gas chromatograph (EC mode) primarily to determine whether the extracts will require further adjustment by dilution or concentration.

2. When appropriate dilution adjustments have been made in the extracts and column oven is set to a known temperature, the relative retention values of the peaks on the chromatograms should be calculated. When these values are compared with the values in the printed table for the appropriate column, the

operator should be able to make tentative compound identifications. Microcoulometry and/or TLC may be required for positive confirmation of some of the suspect chlorinated compounds, whereas FPD may be utilized for the organophosphate suspects.

IX. ALUMINA COLUMN ELIMINATION:

It has been reported by several field scientists analyzing house dust that the alumina cleanup can be bypassed with no ill effects. In view of the expenditure of extra time and material, a laboratory conducting monitoring studies might find it advisable to make some recovery studies eliminating this step by taking the extract mentioned in Step 7 under EXTRACTION AND STARTING THE Florisil fractionation with Step 6 of Subsection VII.

TYPICAL RECOVERY DATA - Soxhlet Method

-Pesticides-

	Lindane	Hep. Epox.	p,p'-DDE	Dieldrin	p,p'-TDE	p,p'-DDT
SOILS:						
Mean percent recovery:	85.25	87.83	83.08	88.25	91.17	94.17
S.D. :	5.446	9.446	6.345	6.210	6.886	8.922
n :	12	12	12	12	12	12
HOUSE DUSTS:						
Mean percent recovery:	87.33	80.58	82.92	86.27	90.00	87.78
S.D. :	6.997	12.85	6.345	12.89	9.715	20.20
n :	12	12	12	11	12	9

ORGANOCHLORINE AND ORGANOPHOSPHORUS INSECTICIDES IN BOTTOM SEDIMENT

I. INTRODUCTION:

The examination of sediment from the bottom of a stream or lake provides information concerning the degree of pollution resulting from pesticides, particularly the organochlorine compounds which are not readily biodegradable. This information combined with residue data obtained by analysis of the water and tissues from resident marine life contribute in the development of a overall profile of the pesticidal contamination of a given body of water.

REFERENCES:

1. Column Extraction of Pesticides from Fish, Fish Food and Mud, Hesselberg, R. J. and Johnson, J. L., Bull. Environ. Contam. Toxicol. 7(2/3), 115-120 (1972).

2. Sediment Extraction Procedure, Southeast Water Laboratory, EPA, Athens, Georgia, Method Number SP-8/71.

II. PRINCIPLES:

The sediment sample is partially dried and extracted by column elution with a mixture of 1:1 acetone/hexane. The extract is washed with water to remove the acetone and then the pesticides are extracted from the water with 15% CH_2Cl_2 in hexane. The extract is dehydrated, concentrated to a suitable volume, subjected to Florisil partitioning, desulfurized if necessary, and analyzed by gas chromatography.

III. EQUIPMENT AND REAGENTS:

1. Pans, approximately 14 in. x 10 in. x 2-1/2 in.

2. Oven, drying.

3. Muffle furnace.

4. Desiccator.

5. Crucibles, porcelain, squat form, size 2.

6. Omni or Sorvall mixer with chamber of ca 400 ml.

7. Chromatographic columns, 300 mm x 22 mm with Teflon stopcock.

8. Separatory funnels, 500 ml and 250 ml with Teflon stopcocks.

9. Filter tube, 180 mm x 25 mm.

10. Kuderna-Danish concentrator fitted with grad. evaporative concentrator tube. Available from the Kontes Glass Company, each component bearing the following stock numbers:

    a. Flask, 250 ml, stock #K-570001.

    b. Snyder column, 3 ball, stock #K-503000.

    c. Steel springs, 1/2 in., stock #K-662750.

    d. Concentrator tubes, 10 ml, size 1025, stock #K-570050.

11. Pyrex glass wool - preextracted with methylene chloride in a Soxhlet extractor.

12. Hot water bath, temp. controllable at 80°C.

13. Sodium sulfate, anhydrous, Baker, prerinsed or Soxhlet extracted with methylene chloride.

14. n-Hexane, pesticide quality.

15. Acetone, pesticide quality.

16. Methylene chloride, pesticide quality.

17. Acetone-hexane, 1:1.

18. Diethyl ether, pesticide quality, free of peroxides.

19. Distilled water, suitable for pesticide residue analysis.

20. Sodium sulfate solution, saturated.

21. Methylene chloride-hexane, 15% v/v.

IV. SAMPLE PREPARATION AND EXTRACTION:

1. Decant and discard the water layer over the sediment. Mix the sediment to obtain as homogeneous a sample as possible and transfer to a pan to partially air dry for about 3 days at ambient temperatures.

NOTE: Drying time varies considerably depending on soil type and drying conditions. Sandy soil will be sufficiently dry in one day, whereas muck requires at least three days. The silt and muck sediment is sufficiently dry when the surface starts to split. But there should be no dry spots. Moisture content will be 50-80% at this point.

2. Weigh 50 gram of the partially dried sample into a 400-ml Omni-Mixer chamber. Add 50 gram of anhydrous sodium sulfate and mix well with a large spatula. Allow to stand with occasional stirring for approximately one hour.

   NOTE: As the final calculations will be made on a "bone dry" basis, it is necessary at this point to initiate the test for percent total solids in the sample being extracted for pesticide evaluation. Immediately after weighing the 50 gram sample for extraction, weigh ca 5 gram of the partially dried sediment into a tared crucible. Determine the percent solids by drying overnight at 103°C. Allow to cool in a disiccator for half an hour before weighing. Determine the percent volatile solids by placing the oven-dried sample into a muffle furnace and igniting at 550°C for 60 minutes. Allow to cool in a desiccator before weighing.

3. Attach the 400 ml chamber to an Omni or Sorvall mixer and blend for about 20 seconds. The sample should be fairly free flowing at this point.

4. Carefully transfer the sample to a chromatographic column. Rinse the mixer chamber with small portions of hexane adding the rinsings to the column.

5. Elute the column with 250 ml of 1 acetone-hexane at a flow rate of 3-5 ml/min into a 400 ml beaker.

6. Concentrate the sample extract to about 100 ml under a nitrogen stream and at a temp. no higher than 55°C. Transfer to a 500 ml separatory funnel containing 300 ml of distilled water and 25 ml of saturated sodium sulfate solution. Shake the separatory funnel for two minutes.

7. Drain the water layer into a clean beaker and the hexane layer into a clean 250 ml separatory funnel.

8. Transfer the water layer back into the 500 ml separatory funnel and reextract with 20 ml of 15% methylene chloride in hexane, again shaking the separatory funnel for two minutes. Allow the layers to separate. Discard the water layer and combine the solvent extracts in the 250 ml separatory funnel.

9. Wash the combined solvent extract by shaking with 100 ml of distilled water for 30 seconds. Discard the wash water and rewash the extract with an additional 100 ml of distilled water, again discarding the wash water.

10. Attach 10 ml evap. concentrator tube to a 250 ml Kuderna-Danish flask and place under a filter comprised of a small wad of glass wool and ca 1/2 inch of anhydrous Na_2SO_4 in a filter tube.

11. Pass the solvent extract through the drying filter into the K-D flask, rinsing with 3 portions of ca 5 ml each of hexane.

12. Attach Snyder column to top joint of K-D flask, immerse tube in 80°C water bath and concentrate extract to 5 ml or to a lesser volume if extremely low concentration levels of pesticides are expected.

V. FLORISIL PARTITIONING:

Remove tube from water bath rinsing joint with a small volume of hexane. The partitioning is carried out as described in Section 11A, starting at VII, Step 6.

VI. GAS CHROMATOGRAPHY:

Again proceed as describe in Section 11A.

VII. CALCULATIONS:

1. Percent Dry Solids

$$\frac{\text{gram of dried sample}}{\text{gram of sample}} \times 100 = \%\ \text{Dry Solids}$$

2. Percent Volatile Solids

gram of dried sample - gram of ignited sample = gram of volatile solids

$$\frac{\text{gram of volatile solids}}{\text{gram of sample}} \times 100 = \%\ \text{Volatile Solids}$$

3. Concentration of Pesticide in Sediment

% dry solids x 5 gram = gram of dry sample extracted

$$\frac{\text{l of sample extract injected}}{\text{l of sample extract}} \times \text{gram of dry sample extracted} = \text{gram of dry sample injected}$$

$$\frac{\text{ng of pesticide}}{\text{gram of dry sample injected}} = \text{ppb of pesticide}$$

VIII. SULFUR INTERFERENCE:

Elemental sulfur is encountered in most sediment samples, marine algae and some industrial wastes. The solubility of sulfur in various solvents is very similar to the organochlorine and organophosphate pesticides; therefore, the sulfur interference follows along with the pesticides through the normal extraction and cleanup techniques. The sulfur will be quite evident in gas chromatograms obtained from electron capture detectors, flame photometric detectors operated in the sulfur or phosphorus mode, and Coulson electrolytic conductivity detectors. If the gas chromatograph is operated at the normal conditions for pesticide analysis, the sulfur interference can completely mask the region from the solvent peak through aldrin.

This technique eliminates sulfur by the formation of copper sulfide on the surface of the copper. There are two critical steps that must be followed to remove all the sulfur: (1) the copper must be highly reactive; therefore, all oxides must be removed so that the copper has a shiny, bright appearance; and (2) the sample extract must be vigorously agitated with the reactive copper for at least one minute.

It will probably be necessary to treat both the 6% and 15% Florisil eluates with copper if sulfur crystallizes out upon concentration of the 6% eluate.

Certain pesticides will also be degraded by this technique, such as the organophosphates, chlorobenzilate and heptachlor (see Table 1). However, these pesticides are not likely to be found in routine sediment samples because they are readily degraded in the aquatic environment.

If the presence of sulfur is indicated by any exploratory injection from the final extract concentrate (presumably 5 ml) into the gas chromatograph, proceed with removal as follows:

1. Under a nitrogen stream at ambient temp., concentrate the extract in the concentrator tube to exactly 1.0 ml.

2. If the sulfur concentration is such that crystallization occurs, carefully transfer, by syringe, 500 μl of the supernatant extract (or a lesser volume if sulfur deposit is too heavy) into a glass-stoppered, 12 ml grad., conical centrifuge tube. Add 500 μl of iso-octane.

3. Add ca 2 µg of bright copper powder, stopper and mix vigorously 1 minute on a Vortex Genie mixer.

   NOTE: The copper powder as received from the supplier must be treated for removal of surface oxides with 6N HNO_3. After about 30 seconds of exposure, decant of acid, rinse several times with dist. water and finally with acetone. Dry under a nitrogen stream.

4. Carefully transfer 500 µl of the supernatant-treated extract into a 10 ml grad. evap. concentrator tube. An exploratory injection into the gas chromatograph at this point will provide information as to whether further quantitative dilution of the extract is required.

   NOTE: If the volume transfers given above are followed, a final extract volume of 1.0 ml will be of equal sample concentration to a 4 ml concentrate of the Florisil cleanup fraction.

TABLE 1. EFFECT OF EXPOSURE OF PESTICIDES TO MERCURY AND COPPER

Compound	Percentage Recovery Based on Mean of Duplicate Tests Mercury	Copper
BHC	81.2	98.1
Lindane	75.7	94.8
Heptachlor	39.8	5.4
Aldrin	95.5	93.3
Hept. Epoxide	69.1	96.6
p,p'-DDE	92.1	102.9
Dieldrin	79.1	94.9
Endrin	90.8	89.3
DDT	79.8	85.1
Chlorobenzilate	7.1	0
Aroclor 1254	97.1	104.3
Malathion, diazinon, Parathion, Ethion, Trithion	0	0

DETECTION OF CARBAMATE PESTICIDES IN SOIL

A method investigation is being conducted on the procedure referenced below. If the procedure proves suitable for inclusion in this Manual, an addendum will be forwarded to all current holders of this latest revision.

REFERENCE:

Direct Gas Chromatographic Determination of Carbamate Pesticides Using Carbowax 20M-Modified Supports and the Electrolytic Conductivity Detector, Hall, R. C., and Harris D. E., J. Chromatogr., 169, 245-259 (1979).

CONFIRMATORY PROCEDURES

INTRODUCTION

Gas Chromatography is primarily a quantitative tool which also provides broad information on the identity of organic compounds. When the gas chromatograph is used with the nonspecific electron capture detection system, additional evidence is often necessary to confirm the identity of resulting peaks.

The nature of our analyses are such that interfering materials and artifacts are often observed and matabolic and decomposition products may be encountered. While it is necessary that low concentrations of pesticide residues be detected and measured, it is essential that every agent reported be correctly identified. Whenever one observes unsymmetrical peaks, or unexpected or unexplainable results, the identity of such peaks should be confirmed. In the absence of this identification, one cannot produce reliable quantitative data since quantitation with electron capture gas chromatography depends entirely on the identity of the agent, due to the variation in response among different pesticides. In addition, it would be impossible to interpret the relationship of pesticides to human health by utilizing unreliable qualitative data.

Thus, in order to provide for this most important identification factor, confirmatory methods are included in this manual. The methods discussed include thin-layer chromatography, infrared spectroscopy, extraction p-values and derivatization techniques.

Since the concentrations of pesticides in human tissue are low, and rigorous cleanup is required, and since the equipment available for confirmation lacks sensitivity, macro sampling is necessary. As indicated previously, however, the determination of p-values may be accomplished with micro-samples.

CONFIRMATION AND DETERMINATION OF ORGANOCHLORINE PESTICIDES IN HUMAN TISSUE AND MILK

I. INTRODUCTION:

The method described in the section makes use of a gas chromatgraph equipped with a Carbowax 20M column and a Hall electrolytic conductivity detector for the determination of chlorinated pesticides in human adipose tissue and human milk samples at concentrations as low as 0.01 ppm. Gel permeation chromatograph is used for additional cleanup of extracts having an adverse effect on the performance of the Hall detector due to excessive lipid material. A high degree of correlation was obtained between results of analyses made with this procedure and those using an electron capture detector. Application of the Hall detector for confirmation of organochlorine pesticides can provide an inexpensive substitute for combined gas chromatography-mass spectrometry in some situations.

REFERENCE:

Application of the Hall Detector and a Surface-Bonded Carbowax 20M Column to Analysis of Organochlorine Pesticides in Human Biological Samples, Crist, H. L., and Moseman, R.F., J. Chromatogr., 160, 49-58 (1978).

II. PRINCIPLE:

Human adipose tissue and human milk samples are extracted and cleaned up by a modified Mills, Olney, Gaither procedure. Additional cleanup of fractions is accomplished with gel permeation chromatography prior to gas chromtographic determination on a Carbowax 20M column with a Hall electrolytic conductivity detector.

III. APPARATUS:

1. Tracor Model 222 gas chromatograph (or equivalent) equipped with a Tracor 700 Hall electrolytic detector (see Section 4,C)
2. GLC column 1.8 m x 4 mm i.d. borosilicate glass, packed with surface bonded Carbowax 20M on Chromosorb W (Section 4,A,(7)). A 2 cm section of 5% Carbowax 20M on Chromosorb W is placed on the injection side of the Carbowax column to protect it from buildup of lipid material.

3. Go-Getter gas purifer for helium carrier gas (General Electric Schenectady, NY; distributed by Alltech, Arlington Heights, IL).

4. Autoprep Model 1001 gel permeation chromatograph (Analytical Biochemistry Laboratories, Columbia, MO) equipped with a 350 mm x 25 mm i.d. glass column containing 60 grams of 200-400 mesh BioBeads SX-3 (see Section 5,B).

5. Equipment needed for modified MOG cleanup procedure (Section 5,A,(1),III).

IV. REAGENTS AND SOLVENTS:

1. Pesticide analytical reference standards, available from the Quality Assurance Section (MD-69), Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC 27711.

2. BioBeads SX-3, 200-400 mesh, Bio-Rad Labs, Richmond, CA.

3. Toluene and ethyl acetate of pesticide grade quality.

4. Reagents needed for modified MOG cleanup procedure (Section 5,A,(1),IV).

5. Glass column, 350 mm x 25 mm i.d., Kontes K-422351, and organic solvent plunger assembly, Kontes K-422353.

V. PROCEDURE:

1. Extract and clean up human adipose tissue and human milk samples by modified MOG procedure in Section 5,A,(1) of this Manual.

2. Carry out additional cleanup on the petroleum ether-diethyl ether (85:15 v/v) fraction from the adipose tissue extracts and the (94:6 v/v) fraction from the human milk extracts by gel permeation chromatograph.

   a. Follow the column preparation and operation procedure described in Section 8,M,j,3 of the EPA Pesticide AQC Manual.

   b. Dissolve the evaporated fractions from the MOG cleanup in toluene-ethyl acetate (1:3 v/v).

   c. Inject samples equivalent to ≤1 gram of fat.

   d. Use toluene-ethyl acetate (1:3 v/v) as the elution solvent with a flow rate of 5 ml/minute.

e. Discard the first 100 ml solvent containing the lipids, and collect the next 95 ml containing the pesticides.

NOTE: The analyst should determine the elution pattern of his GPC column with pesticide standards in order to assure quantitative recovery.

3. Concentrate the elution to an appropriate volume in a Kuderna-Danish concentrator assembly.

VI. GAS CHROMATOGRAPHY:

1. Operate the Hall detector with the following parameters:

Quartz combustion tube	18.3 cm x 2 mm i.d.
Furnace temperature	820°C
Hydrogen flow rate	20-40 ml/minute
Transfer line	270°C
Methanol flow rate through detector cell	0.3-0.5 ml/minute

2. Operate the Carbowax column at 175°C or 185 C° with a helium flow rate of 50 ml/minute.

3. Pesticides eluting in the respective Florisil fractions (Section 5,A,(1), Table 1), such as oxychlordane, transnonachlor, p,p'DDE, and p,p'-DDT in the 6% ether fraction and dieldrin in the 15% fraction, can be determined using the Carbowax 20M column. Extracts can be composited for confirmatory analyses. Figure 1 shows the chromatogram for p,p'-DDE from milk and Figure 2 the chromatogram for dieldrin from adipose tissue.

NOTE: A 5% OV-1 column at 200°C was used for determination of β-HCH because of interference from heptachlor epoxide on the Carbowax 20M column.

VII. RESULTS:

Table 1 gives the results of quantitations of p,p'-DDE in the 6% diethyl ether MOG fraction from milk and dieldrin in the 15% diethyl ether fraction from human adipose tissue, using both the Hall and electron capture detectors. The 6% ether MOG fractions and from nine adipose tissue samples were analyzed by both detectors, and graphical comparison of the data made by plotting the results (ppm) against each other. Regression lines for five different pesticides calculated by the least squares method conformed to a straight line ($y = a + bx$) with coefficients of correlation ranging from 0.895 to 0.984. The relatively close agreement of these data from the Hall detector and the electron capture detector indicate the feasibility of using the former for determining chlorinated pesticides in biological samples at levels as low as 0.01 ppm.

The linearized ^{63}Ni electron capture detector was operated at 275°C with a 230°C transfer line. A 1.5% OV-17/1.95% QF-1 column at 200°C with a methane-argon (5.95% v/v) flow rate of 60 ml/minute was used for determinations by EC GLC.

VIII. DISCUSSION AND MISCELLANEOUS NOTES:

1. Innumerable injections of 6% diethyl ether MOG fractions (12 mg tissue equivalent per injection) were made without observing any deterioration in the Hall detector sensitivity or Carbowax 20M column performance. The glass demister tubes in the injection port of the gas chromatograph were changed daily.

2. When the 15% diethyl ether MOG fractions were analyzed on a routine basis, sensitivity of pesticide detection, column resolution, and peak distortion was highly dependent on the accumulation of lipid residue in the demister tube in the injection port. Frequent installation of the clean demister tube was beneficial in restoring the performance of the GLC system, but if too many injections were made, a new combustion tube and ion exchange resin had to be installed in the Hall detector and the conductivity cell had to be cleaned. Replacing the 2 cm layer of 5% Carbowax 20M and the glass wool plug at the injection end of the column and heating at 230-240°C overnight was also beneficial.

3. Figure 3 indicates the beneficial effect on response of additional GPC cleanup of the 15% diethyl ether MOG fraction from human adipose tissue extract. As many as 20 injections were made during a day without changing the demister trap; after 40 injections, no significant decrease in response or column resolution was observed. Removal of excess lipids by GPC reduced instrument "down-time" and service and allowed much lower levels of pesticides to be detected and quantitated.

TABLE 1. DETERMINATION OF DIELDRIN AND p,p'-DDE IN HUMAN BIOLOGICAL EXTRACTS*

Sample	Amount found (ppm) Electron capture	Hall detector	Difference (%)**
Adipose tissue	0.11	0.12	9
Adipose tissue	0.02	0.03	50
Adipose tissue	0.07	0.09	29
Adipose tissue	0.10	0.08	20
Milk	0.01	0.01	0
Milk	0.04	0.04	0
Milk	0.05	0.04	20
Milk	0.02	0.02	0
Milk	0.04	0.04	0
Milk	0.04	0.03	25
Milk	0.03	0.03	0
Milk	0.08	0.08	0
Milk	0.03	0.04	33

*Adipose tissue was analyzed for dieldrin; milk was analyzed for p,p'-DDE.

**Calculated using the elctron capture result as the accepted reference value. Average difference, 14%.

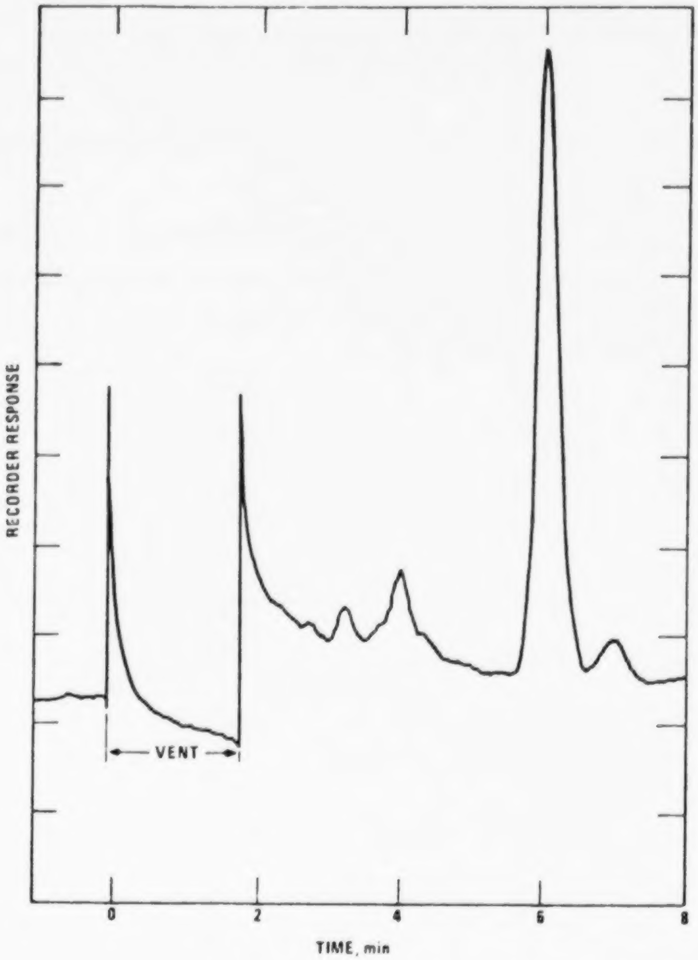

Fig. 1. Chromatogram of 6% fraction from human milk extract after cleanup with GPC (44 ppb p,p'-DDE). Injection: 3 µl/ml (42 mg tissue equivalent); detector: Hall electrolytic conductivity; oven temperature: 185°C; carrier gas flow rate: 50 ml/minute; reaction gas flow rate: 20 ml/minute; furnace temperature: 820°C.

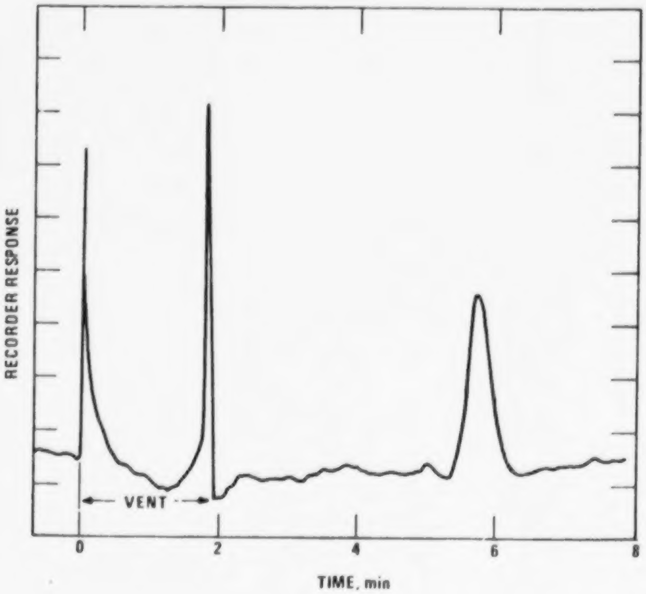

Fig. 2. Chromatogram of 15% fraction from human adipose tissue extract after cleanup with GPC (30 ppb dieldrin); Injection: 5 µl/1.0 ml (13 mg tissue equivalent); detector: Hall electrolytic conductivity; column: Carbowax 20M; oven temperature: 175°C; for other instrument conditions, see Fig. 1.

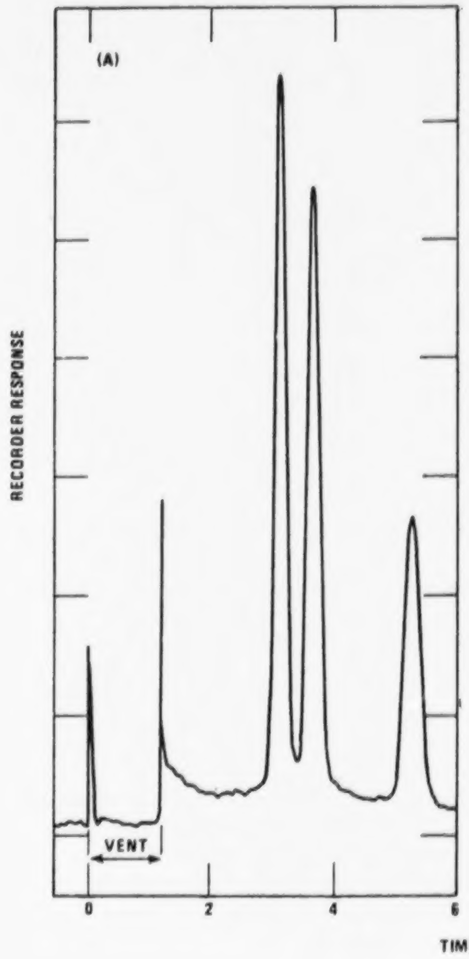

Fig. 3. Chromatograms of (A) pesticide mixture before injection of sample extracts; (B) pesticide mixture after six injections of the 15% fraction from human adipose tissue extracts without GPC (22 mg tissue equivalent/injection). Pesticide mixture = 600 pg oxychlordane, heptachlor epoxide and dieldrin in order of elution. Detector: Hall electrolytic conductivity; oven temperature: 185°C; for other instrument conditions, see Fig. 1.

CONFIRMATORY PROCEDURES

THIN-LAYER CHAROMATORAPHY

I. INTRODUCTION:

Thin-layer chromatography is primarily a qualitative tool which is useful in the identification of pesticides. It can be used to advantage as a confirmatory method in conjunction with gas chromatography. Thin-layer chromatography introduces a second physical basis for separation, that of adsorption chromatography.

Additional advantages of this technique, include simplicity, rapidity, low man-hour consumption, and its utility as a resolving, and cleanup procedure, for use with other methods of analysis.

The method is, in general, somewhat less sensitive than microcoulometry, being limited to about 10 ng for easy visual inspection of most chlorinated pesticides and about 50 ng of most organothiophosphates. Consequently, a macrosample is required. A stringent sample cleanup procedure is also required.

REFERENCES:

1. Kovacs, Martin, F., JAOAC. 46 (1963).
2. Kovacs, Martin, F., Ibid, 47 (1964)
3. Kovacs, Martin, F., Ibid, 48 (1965).
4. Kovacs, Martin, F., Ibid, 49 (1966).
5. Kovacs, Martin, F., Private Communication (1968).
6. Moseman, Robert, Private Communication (1968).
7. Pesticide Analytical Manual, U. S. Food & Drug Administration, Volume I, Sect. 410.

II. APPARATUS:

1. 8" x 8" glass plates, double strength window glass (Pittsburg Plate Glass).
2. 3-1/4" x 4" clinical microslides (Arthur H. Thomas Co.).

3. Developing tank, Thomas-Mitchell, 8-1/2" x 4-1/2" x 8-1/2" deep (Arthur H. Thomas Co.).
4. Desaga/Brinkmann standard counting board.
5. Chromatographic chamber, 800 ml beaker.
6. Desaga/Brinkman standard model applicator.
7. Desaga/Brinkman drying rack, holds 10, 8" x 8" plates.
8. Spotting pipettes, 1, 5, and 10 μl, Kontes 763800.
9. Spray bottle, 8 oz., Thomas Co. #9186-R2.
10. Desaga/Brinkman glass vacuum desiccator.
11. Desk blotter paper.
12. Ultra violet light source: 4-15 watt G. E. germicide lamps, shielded to protect operator, General Electric Co. G 15 T 8.

III. REAGENTS:

1. Aluminum oxide G. (Brinkman or Warner-Chilcott).
2. N-heptane, chromatopgraphic grade.
3. Methycyclohexane, practical, B.P. 100.5 - 101.5°C. (Matheson, Coleman, and Bell).
4. Tetrabromophenolphthalein ethyl ester (Eastman Organic Chemicals #6810).
5. Acetone, Reagent.
6. Silver Nitrate, Reagent
7. Tetraethylenepentamine.
8. Citric Acid, granular, Reagent.
9. p-Nitrobenzyl pyridine.
10. Hydrogen peroxide, 30% Reagent.
11. Ethyl ether, Reagent.
12. Acetonitrile, chromatographic grade.

13. Dimethylformamide, Reagent

14. Preparation of reagent solutions.

   A. Developing solvents.

      (1) For organochlorines

         (a) 2% acetone in N-heptane (v/v) (mobile solvent).

         (b) N-heptane (mobile solvent).

      (2) For thio and nonthio organophosphates.

         (a) Methylcyclohexane (mobile solvent).

         (b) 15% or 20% dimethylformamide (v/v) in ether (immobile solvent).

   B. Chromogenic reagents.

      (1) For organochlorines.

         (a) Dilute 0.1 gm of silver nitrate and 20 ml of 2-phenoxyethanol to 200 ml with acetone. Immediately add 3 drops of 30% hydrogen peroxide and mix. Keep stored in a cool dark place not longer than 1 week. Dark solutions should be discarded.

      (2) For organothiophosphates.

         (a) Stock dye solution.

         Dissolve 1 gm of tetrabromophenolphthalein ethyl ester in 100 ml of acetone.

         (b) Use concentration dye solution.

         Dilute 10 ml of stock solution to 50 ml with acetone.

         (c) Silver nitrate solution.

         Dissolve 0.5 gm $AgNO_3$ in 25 ml of distilled water and dilute to 100 ml with acetone.

         (d) Critic acid solution.

         Dissolve 5 g citric acid in 50 ml of distilled water and dilute to 100 ml with acetone.

(3) For thio and nonthio organophosphates.

(a) 2% p-Nitrobenzyl pyridine in acetone (w/v).

(b) 10% Tetraethylenepentamine in acetone (v/v).

IV. PREPARATION OF TLC PLATES:

8" x 8" plates

1. Add 30 g aluminum oxide G to 50 ml distilled water and shake for 30-45 seconds.

2. Pour into applicator and spread a 250 micron layer on glass plates. Make an arrow in corner of plate indicating direction of application.

3. Air dry for 15 minutes, then at 80°C for 45 minutes in a forced draft oven.

4. Remove, cool, and store plates in a desiccator.

3-1/4" x 4" micro slide plates

1. Preparation of adsorbent layer - Select five 8" x 8" and one 4" x 8" (calculated to cover the entire surface of the applicator board) photo-glass plates of uniform width and thickness. Wet the surface of the applicator board with a few ml of distilled water delivered from an eye dropper in the form of the letter "X", approximately the size of the plate to be mounted. Press each plate snugly into position to ensure a tight fit. (Add enough water to the applicator board to prevent the appearance of air bubbles under the plate after it has been pressed into position.

2. Examine each 3-1/3" x 4" micro slide carefully by looking down each edge. To ensure flatness of plates, use only slides that are visibly straight along all edges.

3. Mount the micro slides individually on the surface of the photoglass plates with their long axis perpendicular to the direction of layer application. With an eye dropper, place a few ml distilled water on the surface of the photoglass plate and mount micro slides. Force out the excess water so that no large air bubbles remain under the slide. The presence of a large air bubble indicates slide "bowing" due to an irregularity in the slide. When "bowing" is noted, discard the slide, and position another in its place.

4. Repeatedly slide an empty applicator across the series of mounted slides to force out excess water, and wipe surface of slides dry each time with a tissue. The empty applicator must ride smoothly and without effort across the series of slides. If not, reexamine the uniformity and positioning of micro slides.

5. To remove any remaining water, wipe the surface with a dry tissue, then with one soaked in 95% ethanol, and let dry.

6. Weigh 30 g Al_2O_3 G of MN-silica gel G-HR into a 250 ml ~~3~~ Erlenmeyer flask. Add 50 ml distilled water to Al_2O_3 G or 60 ml to MN-silica get G-HR, stopper flask, shake moderately for 15 to 20 seconds, and immediately pour slurry into applicator chamber. The time required for actual application should be approximately 10 seconds. Immediately after coating, grasp the applicator board at its ends, raise a few inches, then drop. This procedure which is repeated a few times, smooths out slight ripples or imperfections in the wet coating.

7. Let coated plates dry in position on the mounting board for 20 minutes. Mark each micro plate on the 3-1/4" edge farthest from the longitudinal center of the applicator board. This edge represents the top of each micro plate during subsequent development. The 3-1/4" edge of each micro plate at the center of the applicator board represents the end to be spotted. This is done because most of the coating irregularity occurs on the outer 3-1/4" edge of the plates.

8. Remove each plate individually with a spatula, and wipe the back side dry with a tissue. Place 4 micro slides on the surface of one 8" x 8" plate and place the plates in a rack for drying at 80°C for 1 hour in a forced draft oven.

9. After heating, cool the micro plates, and examine each individually in strong transmitted light for possible gross irregularities in the uniformity of the coating. Discard plate if gross irregularities are observed. Place 4 micro plates on the surface of each 8" x 8" plate, slide into a drying rack, and store in a desiccating storage cabinet until needed.

10. Sample spotting - Make a pencil mark at each side 1/2" above the bottom edge of the slide. The imaginary line between these points serves as the sample "spotting line." Draw an actual line across the slide 2-3/8" (about 6 cm) above the "spotting line." The actual line serves to mark the solvent front after development. Draw a pencil line along each side 1/4" in from the edge to prevent distortion of the solvent front during development.

11. Spot samples at 1/4" intervals along the imaginary "spotting line." Each micro plate will accommodate 10 application points as compared to 18 on a normal 8" x 8" plate. Spot samples and standards on the micro slide in the same manner as described later under SPOTTING.

V. PRECOATED PLATES:

The laboratory which conducts TLC constantly would probably find it more economical to purchase the coating equipment and prepare their own plates. Many smaller laboratories, however, which may conduct TLC only occasionally as a confirmation technique will probably find it more convenient to use precoated plates from commercial suppliers.

There are a number of high quality competitive brands of precoated plates in the marketplace. Two sources which are known to the editor to market suitable plates are:

(1) Brinkman Instruments Inc., Westbury, N.Y. 11590
Aluminum oxide precoated TLC sheets - aluminum oxide F-254 neutral (Type E) on aluminum, 20x20 cm, Merck-Darmstadt, Cat. No. 68 23 050-1.

(2) Quantum Industries, 341 Kaplan Drive, Fairfield, NJ 07006
Aluminum oxide TLC plates, 20x20 cm, type Q3, Code 1023, 25 plates per package.

Plates that incorporated the $AgNO_3$ in the precoated Al_2O_3 layer should not be used where sensitivity is a factor.

VI. SAMPLE PREPARATION:

1. The sample must be of sufficient size that when the extract from Florisil cleanup is concentrated to an appropriate volume, a 10 µl spotting volume will produce detectable compound spots. A serum extract from 50 grams concentrated to 100 µl should produce a visible spot of 2 ppb. An adipose tissue extract from 5 grams concentrated to 500 µl should give a readable spot at 10 ppb. These values assume the detection of chlorinated pesticides.

2. The extract from the 15% diethyl ether fraction contains far more lipids than are present in the 6% fraction. For this reason, some further cleanup is required. This is conveniently accomplished by spotting the equivalent of 5 grams of blood or 0.5 grams of fat on a 3-1/4" x 4" micro plate, developing with acetonitrile, and scraping off the alumina from the area at the solvent front. This is extracted in hexane and the resulting extract respotted on a standard 8" x 8" TLC plate.

VII. SPOTTING AND DEVELOPING:

1. Provide an imaginary spotting line across the plate by making a pencil mark 1-1/2" from the bottom edge of the plate on both sides.
2. Provide an imaginary solvent front by making a pencil mark 5-1/2" from the bottom edge of the plate on both sides.
3. With a micropipette, transfer a suitable amount of the extract to one of the spots, with repeated applications.
4. Spot standards solutions on the same plate. Standard concentrations should bracket the calculated amount of residue in sample.
5. Prepare chromatographic tank by placing 50 ml of developing solvent in the trough and 75 ml in the bottom of the tank.
6. Seal the tank and develop to the line scribed on the plate.
7. Remove plate and air dry in the hood.

VIII. COMPOUND DETECTION:

1. Organochlorines
   a. Immediately after drying, spray plates with the chromogenic reagent.
   b. Air dry plates for 15 minutes.
   c. Expose plates to ultraviolet until the lowest concentrations of standards are visible.
2. Organothiophosphates
   a. Immediately after drying, spray plates with the "use concentration" dye solution. Spray moderately heavy.
   b. Overspray lightly with the silver-nitrate solution.
   c. After 2 minutes overspray the plate moderately with citric acid solution.

3. Thio and nonthio organophosphates.

   a. After drying, spray plate with p-Nitrobenzyl pyridine chromogenic solution and heat at 110°C for 10 minutes.

   b. Cool and overspray plate with tetraethylenepentamine solution.

   NOTES:

   1. The color of solid p-Nitrobenzyl pyridine should be yellow. If there is any purple color, recrystallize from acetone. Oxidized solution will cause high background color and will reduce sensitivity.

   2. The tetraethylenepentamine should not have a deep color. If it does, decolorize and purify with charcoal.

4. Interpret results by comparing R_f values of sample spots against those of standard spots on the same plate.

IX. GAS CHROMATOGRAPHY (EC) CONFIRMATION OF R.F. VALUES:

At times there may be reason to question the validity of a spot because of a slight shift in the position of the R. F. site or because of spot diffusion or a very faint appearing spot. When such doubt exists, EC GLC examination of the material from the questionable R. F. site can serve to either confirm or negate the presence of the suspected compound.

Any of the pesticidal compounds (organochlorine) of lowest concentration in blood or fatty tissue such as β-BHC, heptachlor epoxide o,p'-DDT and p,p'-DDD are frequently the most difficult to identify by customary EC GLC. These compounds, if present, are generally in such low concentration that an extract aliquot equivalent to 5.0 grams of blood and 0.5 grams of fat is needed for spotting on the TLC plates.

1. Spot the TLC plate with 200 nanograms each of standards of the suspect compounds. Also spot the 6% diethyl ether fraction of the unknown extract in two places on the plate.

2. Develop the plate with n-Hexane until the solvent front has migrated 10 cm.

3. Cover with a small glass plate a portion of the plate containing one of the sample applications and spray the plate with the $AgNO_3$/2-phenoxyethanol reagent.

4. Develop the spots in the sprayed area in the usual manner and compute the R_f values for the standards.

5. Utilizing the standard R_f values, pin-point the elution sites for these compounds along the imaginary migration line of the unsprayed sample.

6. Scrape each sample elution with a flat edge spatula and transfer the alumina to separate centrifuge tubes.

   NOTE: Scrape another spot from an area of the plate lying outside the region of samples and standards and extract identically to the sample spots. This will serve as a reagent blank.

7. Add 1 ml of hexane which has been previously examined for assurance that it is free of contaminants which might contribute artifact peaks.

8. Stopper tube and shake vigorously one minute on a Vortex mixer.

9. Inject 5 μl of this extract into the gas chromatograph and observe the chromatogram for the presence or absence of the suspect compound peak. Adjustment of the injection volume may be required based on peak height resulting from the initial injection, provided of course, that there are any peaks.

X. MISCELLANEOUS NOTES:

1. Thorough sample and extract cleanup must be employed

2. Plates must be thoroughly washed.

3. All solvents, except ethyl ether, must be redistilled.

4. Prevent even minor contamination.

5. Isooctane tolerates more oil in the sample than other developing solvents.

6. For the detection of nanogram quantities it is imperative to use a source of U. V. radiation at least as intense as that provided by the specified equipment.

7. Always spray the chromogenic agent in a direction perpendicular to the direction of solvent flow (side to side).

n-HEPTANE SOLVENT SYSTEM

Adsorbent	Al O G (Merck), 250 μ thick, air dried 72 hr at room termperature
Plate Size	8"x8"
Front Travel	10 cm
p,*p*'-TDE Travel	3.9 cm
Developing Tank	9"x9"x3.5", saturated
Visualization	$AgNO_3$, UV exposure
Temperature	24-26°C
Amount Spotted	80-200 ng

Pesticide	R_{TDE}
Hexachlorobenzene	2.7
Aldrin	2.1
p,*p*'-DDE	2.0
Heptachlor	2.0
Chlordane (tech)	2.0, 1.8, 1.4, 1.2*
o,*p*'-DDT	1.9
PCNB	1.8
Perthane olefin	1.8
p,*p*'-TDE olefin	1.8
TCNB	1.7
Telodrin	1.7
Toxaphene	1.7, 1.2*
Strobane	1.7, 1.2*
p,*p*'-DDT	1.6
o,*p*'-TDE olefin	1.6
Chlorbenside	1.3 (grey)
BHC (tech)	1.3, 1.1, 0.27, 0.10
α-BHC	1.3
Pethane	1.3
Lindane	1.1
o,*p*'-TDE	1.1
p,*p*'-TDE	1.0
Endosulfan	0.88, 0.07
Ronnel	0.85
Heptachlor epoxide	0.71
Endrin	0.71
Dieldrin	0.52
Carbophenothion	0.42 (yellow)
Methoxychlor	0.33, 0.27
β-BHC	0.27

Pesticide	R_{TDE}
Ovex	0.18
Dichlone	0.16
Dyrene	0.15 (Grey)
Tetradifon	0.11
δ-BHC	0.10
Delta Keto "153"	0.09
Kelthane	0.06
Sulphenone	0.00 (large and fuzzy)
Captan	0.00 (sharp edged grey)
Chlorobenzilate	0.00 (light)
Monuron	0.00 (light and dark)
Diuron	0.00
Endrin aldehyde	0.00 (very small)
Endrin alcohol	0.00

Most intense spot underlined
*Leaves a streak with these major spots

2% ACETONE IN *n*-HEPTANE SOLVENT SYSTEM

Adsorbent	Al_2O_3 G (Merck, 250 µ thick, air dried 72 hr at room temperature
Plate Size	8"x8"
Front Travel	10 cm
p,p'-TDE Travel	5.7 cm
Developing Tank	9"x9"x3.5", saturated
Visualization	$AgNO_2$, UV exposure
Temperature	24-26°C
Amount Spotted	80-200 ng

Pesticide	R_{TDF}
Hexachlorobenzene	1.7
Perthane olefin	1.4
PCNB	1.4
Aldrin	1.4
p,*p*'-DDE	1.4
Chlordane (tech)	1.4, 1.3, 1.2, 1.1*
p,p'-TDE olefin	1.4
Telodrin	1.4
Heptachlor	1.4
TCNB	1.3
o,*p*'-TDE olefin	1.3
Toxaphene	1.3, 1.2*
Strobane	1.3, 1.2*
o,*p*'-DDT	1.3
p,*p*'-DDT	1.2
Chlordenside	1.2 (fuzzy grey)
Perthane	1.2
BHC (tech)	1.1, 0.92, 0.72, 0.25
α-BHC	1.1
Ronnel	1.1
Endrin	1.0
Carbophenothion	1.0 (fuzzy yellow)
Heptachlor epoxide	1.0
p,*p*'-TDE	1.0
o,*p*'-TDE	0.95
Lindane	0.92
Endosulfan	0.92, 0.24
Dieldrin	0.90
Tetradifon	0.82

Pesticide	R_{TDF}
Methoxychlor	0.79
Ovex	0.76
β-BHC	0.72
Dichlone	0.72, 0.00
Dyrene	0.51
Sulphenone	0.31
Kelthane	0.28
δ-BHC	0.25
Delta Keto "153"	0.23 (very small)
Captan	0.09
Chlorobenzilate	0.05
Monuron	0.05
Diuron	0.00 (dark spot)
Endrin Aldehyde	0.00 (very small)
Endrin alcohol	0.00

Most intense spot underlined
*Leaves a streak with these major spots

R_f Values

Adsorbent Mobile solvent	Al_2O_3G Methylcyclohexane	
Pesticide	R_f Value	
	Immobile Solvent	
	15% DMF	20% DMF
Dimethoate	0.01	0.01
Azinphosmethyl (Guthion)	0.09	0.06
Imidan	0.09	0.07
Methyl parathion	0.17	0.11
Coumaphos	0.23	0.15
Malathion	0.34	0.22
Dioxathion	0.37	0.24
Parathion	0.41	0.27
Demeton (thiol)	0.44	0.32
EPN	0.49	0.33
Methyl carbophenothion	0.50	0.36
Sulfotepp	0.69	0.55
Carbophenothion	0.74	0.59
Ronnel	0.76	0.62
Ethion	0.77	0.63
Demeton (thiono)	0.79	0.67
Phorate	0.81	0.71
Disulfoton	0.82	0.72
Diazinon	0.86	0.78

(1) Presence of chloride in adsorbent layer reacts with $AgNO_3$ and prevents coupling of dye and pesticide to form characteristic blue or purple spot. Some aluminum oxide coatings do not have to be prewashed to remove chloride. If, however, maximum compound sensitivities of 0.05 μg cannot be achieved with unwashed Al_2O_3 coating, prewashing is recommended.

(2) Chromogenic spray reacts only with sulfur-containing phosphate esters. The following compounds do not react; oxygen analog of parathion, dichlorvos, naled, mevinphos, Phosphamidon and trichlorfon.

(3) The following minimum amounts of sulfur-containing phosphate esters can be detected: 0.05 μg diazinon, demeton (thino), carbophenothion, parathion, malathion, ronnel, dioxathion, EPN, coumaphos, sulfotepp, and ethion; 0.1 μg azinphosmethyl, methyl parathion, and demeton (thiol). The lower limits of detectability of dimethoate, Imidan, methyl carbophenothion, phorate, and disulfoton were not determined.

At 0.5 μg, or greater, the thio-phosphate esters vary as to color produced with the chromogenic reagents. Carbophenothion, parathion, EPN, coumaphos and diazinon appear vivid blue. Ethion, azinphos-methyl, sulfotepp, diozathion, and malathion appear purple. Ronnel and methyl parathion appear dull blue while both thiol and thiono demeton appear bluish purple.

CONFIRMATORY PROCEDURES

EXTRACTION p-VALUES

I. INTRODUCTION:

The information contained in this section is taken from the FDA PESTICIDE ANALYTICAL MANUAL, Volume 1, based on the original work of Bowman and Beroza (JAOAC, 48, 943, 1965). This system is described as a method of identifying or confirming identity of pesticides at nanogram or other levels of analysis through the use of extractions p-values. The p-value, determined by distributing a solute between equal volumes of two immiscible phases, is defined as the fraction of the total solute partitioning into the upper phase. The value may be derived from a single distribution between the phases or from a multiple distribution as in countercurrent distribution, (2,3). As a single distribution the p-value may be determined easily and rapidly; it is especially useful for confirming the identity of pesticide residues at levels amenable to quantitative analysis by electron capture gas chromatography.

The p-values for 88 pesticides and related compounds in six binary solvent systems are listed in Table 1. These are arranged according to generally ascending values for retention, relative to aldrin. The RR values, where available for the prescribed GLC column are given.

II. EQUIPMENT AND REAGENTS:

1. Gas chromatograph with electron capture detector, equipped with 6' x 1/4" o.d. glass column of 1.5% OV-17/1.95% QF-1

2. Grad. centrifuge tubes, 10 ml, with Ŧ glass stoppers.

3. Solvent systems: Use pesticide quality solvents. To remove interferences, extract distilled water with hexane; reflux hexane, heptane, and 2,2,4-trimethylpentane over sodium hydroxide and distill before use. Equiliberate solvent pairs overnight in a room maintained at 25.5 ± 0.5°C before use. The six solvent systems used in this study are shown in Table 1. Make dilutions of the lower phase with water on a volume basis.

III. PROCEDURE:

The analyses are made by electron capture gas chromatography; 88 compounds were analyzed in this manner.

1. Pipet 5 ml of the hexane (or upper layer) extract into a 10 ml centr. tube, and chromatograph 5 µl.
2. Pipet 5 ml of the opposing solvent (lower layer) into the centr, tube, stopper, and shake vigorously 1 minute.
3. Allow layers to separate and chromatograph 5 µl of upper layer extract.

The p-value is the ratio of the second analysis (amount in upper layer) to the first (total amount). It is reported in hundredths except for values below 0.10 which are reported in thousandths.

IV. SPECIFICITY:

Figure 1 depicts graphically the number of pesticides and related compounds falling at p-value intervals of 0.02 for the six solvent systems. If one depends solely on p-values for identification, specificity of a given p-value will be inversely proportional to the number of possibilities and will increase with the accuracy of the analysis (an error of 0.03 would bring in more possibilities than one of 0.02). Specificity can be increased by determining more p-values, as this process imposes additional criteria on identification. It is also apparent that the more complete the compilation of pesticide p-values, the more reliably can one assess the specificity of a given p-value. The accumulation of p-values at the lower end of the hexane-acetonitrile and the 2,2,4-trimethylpentane-DMF (deimthylformamide) scales of Fig. 1 indicates that a p-value in this range has poor specificity (too many possibilities). Between 0.30 and 0.91 the specificity becomes very good because the number of possibilities at each p-value are few. Thus, by inspecting Figure 1, one can arrive at a decision as to the degree of specificity for a given p-value in a given solvent system. By the same reasoning, the systems heptane-90% ethanol, and 2,2,4-trimethylpentane-80% acetone (latter below 0.72), appear to be more generally useful for identifications than the hexane-acetonitrile and the 2,2,4-trimethylpentane-DMF systems. However, for a specific case, sweeping generalizations as to the best system cannot be made.

Since three-quarters of the p-values are below 0.21 in the hexane-acetonitrile system and the nonpolar crop interference--as from butter--tends to accumulate at higher p-values (2), pesticides are readily separated from such crop interferences by simple extraction. The result illustrates graphically why this solvent system has become popular in pesticide analysis. 2,2,4-trimethylpentane-DMF also appears to be good for such separations, but DMF, boiling about 70° higher than acetonitrile, is much more difficult to evaporate and accordingly less suitable for cleanup

V. COMMENTS:

With the p-value technique it is not usually necessary to determine the exact amount of a substance in an analysis, but only the relative amounts present in the original and the extracted solution. This feature is especially welcome in gas chromatographic analysis when one is dealing with an unknown compound, and the response for a given amount of compound is not known. In such cases, it is desirable to check the linearity of the system by injecting an amount necessary to give a reasonable response and then injecting exactly half that amount. If the second response is half the first, the linearity of the system may be considered satisfactory. This type of linearity check was routinely made in the present work.

In a few instances there appears to be a reaction that takes place between the solute and the solvent system. The reaction either progresses with the time of exposure to the solvent system or may result from the reaction of solvent and solute when they are injected into the hot injection port during gas chromatographic analysis. Some compounds (supposedly pure) give multiple peaks indicating breakdown. The p-values derived from these analyses must be considered less reliable than those of comounds chromatographing without breakdown.

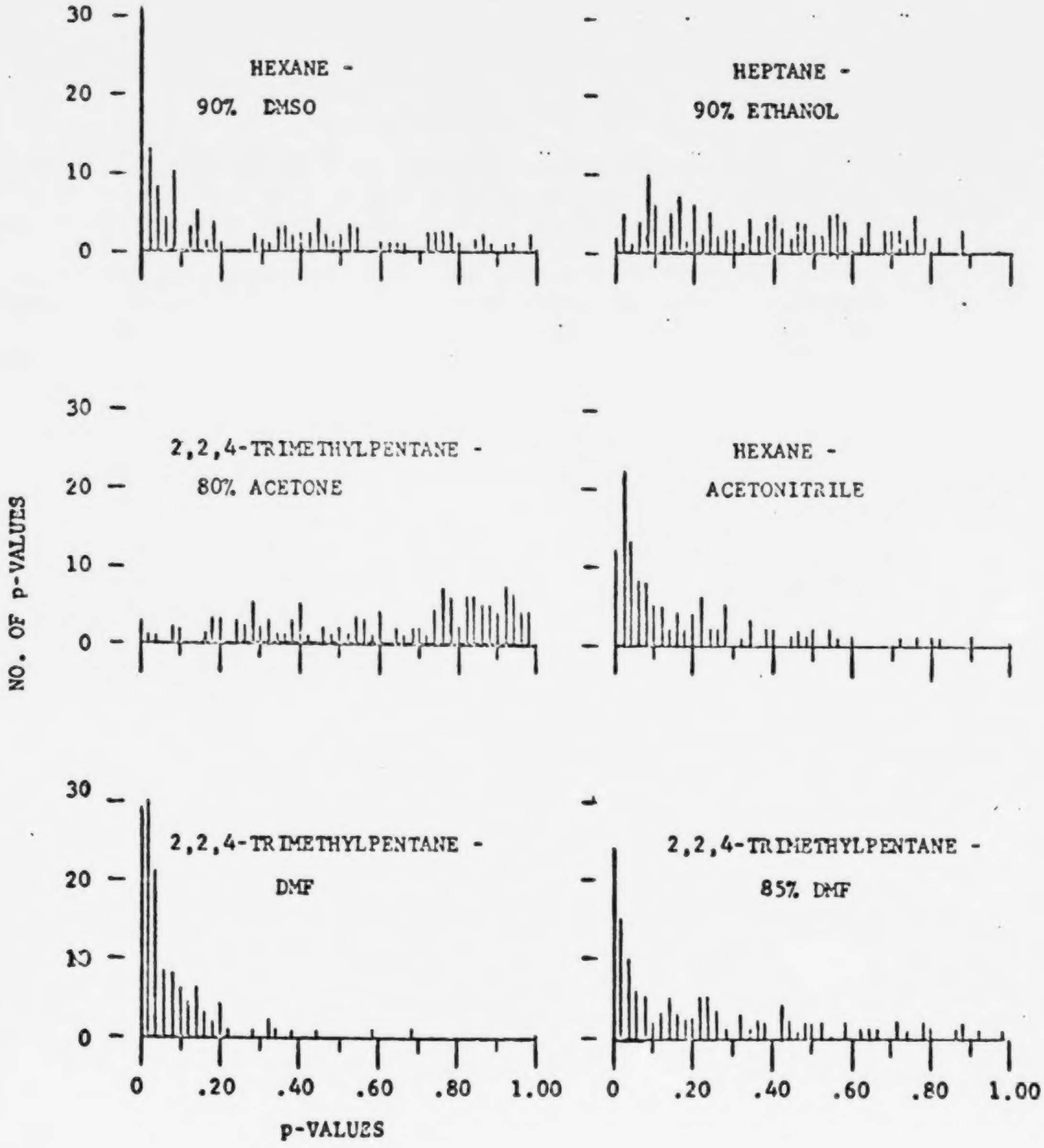

Figure 1. Incidence of p-values in each of the six binary solvent systems.

TABLE 1. P-VALUES OF PESTICIDES AND RELATED COMPOUNDS DETERMINED BY SINGLE DISTRIBUTIONS BETWEEN IMMISCIBLE PHASES AT 25.5 ± 0.5°C ARRANGED ACCORDING TO ASCENDING GAS CHROMATOGRAPHIC RETENTION TIMES -(R_t). COLUMN OF 1.5% OV-17/1.95% QF-1 OPERATED AT 200°C.

			Solvent System					
Compd. No.	Pesticide (Or Related Compound)	R_t (relative to R_t of Aldrin)	Hexane: Acetonitrile	2,2,4-trimethylpentane: DMF	2,2,4-trimethylpentane: 85% DMF	Hexane: 90% DMSO	Heptane: 90% Ethanol	2,2,4-trimethylpentane: 80% Acetone
1	naled		0.12	(a)	(a)	0.085	0.23	0.39
2	ethylene dibromide		0.29	(a)	(a)	0.48	0.58	0.76
3	Fumazone®		0.23	0.12	0.32	0.36	0.54	0.76
4	Penphene®		0.76	0.58	0.89	0.98	0.79	0.87
5	dichlobenil		0.11	0.080	0.15	0.19	0.26	0.60
6	Zinophos®		0.058	0.036	0.23	0.15	0.16	0.40
7	barban		0.019	0.003	0.007	0.003	0.13	0.37
8	chloro-IPC		0.19	0.14	0.17	0.16	0.26	0.72
9	CDEC	0.56	0.22	0.13	0.32	0.35	0.46	0.86
10	phorate	0.52	0.26	0.11	0.44	0.61	0.56	0.83
11	Shell SD 8447 (hydr. prod.)		0.18	0.077	0.24	0.18	0.42	0.85
12	trifluralin		0.23	0.21	0.81	0.84	0.72	0.93
13	lauseto neu		0.023	0.007	0.010	0.008	0.077	0.34
14	lindane	0.69	0.12	0.052	0.14	0.093	0.41	0.78
15	PCNB		0.41	0.23	0.67	0.79	0.82	0.95
16	Bayer 30911		0.23	0.071	0.24	0.33	0.49	0.79
17	dioxathion (primary peak)		0.068	0.038	0.12	0.21	0.39	0.95
18	Stauffer N-2790		0.21	0.081	0.33	0.44	0.48	0.79
19	diazinon	0.64	0.28	0.18	0.52	0.75	0.39	0.75
20	dichlone	0.99	0.073	0.027	0.068	0.068	0.34	0.57
21	Di-Syston®		0.16	0.089	0.36	0.47	0.54	0.82
22	endosulfan ether		0.29	0.14	0.42	0.43	0.45	0.85
23	Bayer 38156		0.22	0.12	0.39	0.51	0.48	0.76
24	Hercules 426		0.50	0.20	0.72	0.79	0.74	0.98
25	heptachlor	0.82	0.55	0.21	0.73	0.77	0.71	0.96
26	methyl parathion	1.45	0.022	0.012	0.015	0.015	0.11	0.40
27	dioxathion (secondary peak)		0.11	0.055	0.25	0.44	0.35	0.81
28	butonate		0.013	^{b}0.005	^{b}0.014	^{c}0.078	0.043	0.080
29	Bayer 41831		0.036	0.016	0.046	0.074	0.24	0.55
30	malathion	1.63	0.042	0.015	0.037	0.077	0.14	0.46
31	Zytron®		0.12	0.058	0.14	0.12	0.35	0.79
32	fenson		0.048	0.013	0.032	0.035	0.20	0.61
33	aldrin	1.00	0.73	0.38	0.86	0.89	0.76	0.98
34	1-hydroxychlordene	1.25	0.068	0.026	0.062	0.033	0.15	0.56
35	Bayer 25141		0.82	0.32	0.78	0.81	0.77	0.90
36	parathion	1.84	0.044	0.029	0.082	0.094	0.30	0.76

(Continued)

TABLE 1. CONTINUED

			Solvent System					
Compd. No.	Pesticide (Or Related Compound)	R_t (relative to R_t of Aldrin)	Hexane: Acetonitrile	2,2,4-trimethylpentane: DMF	2,2,4-trimethylpentane: 85% DMF	Hexane: 90% DSMO	Heptane 90% Ethanol	2,2,4-trimethylpentane: 80% Acetone
37	Dimite®		0.25	0.077	0.27	0.37	0.47	0.81
38	Kelthane®		0.15	0.043	0.18	0.029	0.32	0.84
39	dicapthon		0.031	0.019	0.044	0.051	0.25	0.61
40	Chlorthion®		0.026	0.013	0.039	0.032	0.16	0.56
41	chlorobenzilate (secondary peak)		0.22	0.062	0.24	0.40	0.38	0.93
42	dicryl		0.040	0.029	0.041	0.012	0.066	0.31
43	Telodrin®		0.48	0.17	0.63	0.65	0.73	0.94
44	Bayer 37289		0.54	0.18	0.75	0.78	0.72	0.88
45	isodrin		0.60	0.28	0.78	0.86	0.76	0.97
46	Dyrene®	1.83	0.041	(a)	(a)	0.014	0.17	0.61
47	heptachlor epoxide	1.54	0.29	0.10	0.39	0.35	0.57	0.89
48	Morestan®		0.34	0.14	0.43	0.53	0.54	0.65
49	folpet	2.64	0.066	0.015	0.036	0.025	0.23	0.51
50	Ruelene®		0.031	0.012	0.013	0.012	0.11	0.21
51	γ-chlordane		0.40	0.14	0.48	0.45	0.56	0.95
52	Genite 923®		0.08	0.032	0.076	0.093	0.30	0.67
53	Sulphenone®		0.023	0.012	0.009	0.013	0.087	0.32
54	chlorbenside	1.91	0.24	0.039	0.21	0.29	0.52	0.86
55	endosulfan (I)	1.95	0.39	0.16	0.52	0.55	0.64	0.93
56	ovex		0.068	0.024	0.061	0.053	0.28	0.69
57	Shell SD-8447		0.051	0.038	0.051	0.044	0.093	0.47
58	dieldrin	2.40	0.33	0.12	0.46	0.45	0.54	0.88
59	p,p'-DDE	2.23	0.56	0.16	0.65	0.73	0.76	0.96
60	endrin	2.93	0.35	0.15	0.51	0.52	0.59	0.92
61	endosulfan (II)	3.59	0.13	0.060	0.14	0.093	0.34	0.82
62	Aramite®		0.13	0.075	0.23	0.30	0.43	0.85
63	Methyl Trithion®		0.075	0.019	0.075	0.081	0.42	0.82
64	Perthane®	2.71	0.26	0.077	0.44	0.46	0.70	0.93
65	endrin aldehyde		0.082	0.041	0.083	0.053	0.15	0.79
66	TDE	3.48	0.17	0.038	0.15	0.081	0.46	0.89
67	o,o'-DDT		0.45	0.10	0.42	0.53	0.62	0.91
68	chlorobenzilate (primary peak)		0.14	0.032	0.12	0.14	0.28	0.76
69	o,p'-DDT	3.16	0.47	0.11	0.51	0.66	0.68	0.96
70	Kepone®	2.77	(e)	(e)	(e)	(e)	0.16	0.43
71	Neotran® (primary peak)		0.47	0.11	0.59	0.73	0.77	0.93
72	ethion	4.28	0.079	0.045	0.20	0.38	0.41	0.83

(Continued)

TABLE 1. CONTINUED

Compd. No.	Pesticide (Or Related Compound)	R_t (relative to R_t of Aldrin)	Solvent System: Hexane: Acetonitrile	2,2,4-trimethylpentane: DMF	2,2,4-trimethylpentane: 85% DMF	Hexane: 90% DMSO	Heptane: 90% Ethanol	2,2,4-trimethylpentane: 80% Acetone
73	Prolan®		0.050	0.017	0.048	0.029	0.25	0.75
74	endosulfan sulfate		0.035	0.015	0.023	0.010	0.16	0.68
75	Rhodia R.P. 11783		0.019	0.006	0.012	0.01	0.16	0.38
76	carbophenothion	4.56	0.21	0.037	0.27	0.35	0.56	0.90
77	p,p'-DDT	4.18	0.38	0.084	0.36	0.40	0.64	0.93
78	Bulan®		0.10	0.024	0.10	0.072	0.36	0.86
79	endrin Δ-keto compound		0.10	0.052	0.077	0.062	0.21	0.76
80	Geigy G-28029		0.29	0.065	0.43	0.43	0.64	0.91
81	EPN		0.38	0.011	0.033	0.046	0.24	0.71
82	dinocap (primary peak)[f]		0.092	0.049	0.27	0.54	0.50	0.98
83	methoxychlor	8.1	0.069	0.023	0.092	0.12	0.44	0.74
84	mirex	6.1	0.91	0.33	0.98	0.93	0.88	0.99
85	tetradifon	10.9	0.10	0.041	0.13	0.13	0.40	0.78
86	Guthion®	15.0	0.008	0.002	0.003	0.003	0.14	0.18
87	dinocap (secondary peak)		0.082	0.041	0.22	0.50	0.48	0.94
88	coumaphos		0.006	0.002	0.010	0.013	0.083	0.59

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[a] Solvent interferes with GLC zones under these conditions.

[b] Reduced initial response (reaction with system?). Dyrene response continues to diminish on standing.

[c] Converted to substance $R_t = 0.70$.

[d] R_t changes after equilibration (reaction with system?).

[e] p-Values differ at different concentrations of analysis.

[f] Actually two zones emerging as one.

MICRO SCALE ALKALI TREATMENT FOR USE IN PESTICIDE RESIDUE CONFIRMATION AND SAMPLE CLEANUP

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subsequently published in the Bull. Envir.
Contam. & Toxic., 7,2/3, 160, 1972)

Procedures involving alkali treatment for dehydrochlorination of certain organochlorine pesticides and saponification of fats have long been employed in pesticide residue analysis. In 1942 Brand and Busse-Sunderman (1), and in 1946 Soloway et al. (2), studied rates of dehydrochlorination of DDT. Milles (3) used refluxing alcoholic KOH in the cleanup of fatty foods for paper chromatographic detection of alkali-stable organochlorine pesticides. Klein and Watts (4) used alcohol NaOH to dehydrochlorinate o,p'- and p,p'-DDT, p,p'-TDE, and Perthane prior to gas chromatographic separation of the respective olefins. These investigators called attention to several earlier uses of alkali dehydrochlorination in pesticide residue chemistry. Recent literature contains numerous references to application of this treatment in pesticide residue analyses, including an adaption for use in a pre-gas chromatographic (GLC) column (5).

In spite of the knowledge of this reaction, we have observed that it is not fully and effectively utilized by residue laboratories for forming derivatives, gaining information for identity confirmation, or obtaining better cleanup of troublesome extracts. This is probably because the procedure has not been described in detail for simple micro scale application in multi-residue analysis.

The purpose of this work was (1) to arrive at optimum and convenient parameters of the alkali treatment in order to obtain rapid dehydrochlorination resulting in product solutions suitable for analysis by GLC; (2) to obtain yield and recovery of olefins from a number of bis(phenyl) chloroethane pesticides; (3) to determine the effect of the treatment on several important pesticide and industrial chemicals; (4) to describe in detail the procedure for routine application in the residue analytical laboratory.

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METHOD

Reagents and Apparatus

(a) Potassium hydroxide - Anhydrous pellets.
(b) Ethanol - USP 95%
(c) Hexane - Suitable for use with electron capture gas chromatography (Burdick and Jackson Laboratories, Inc. 1953 S. Harvey St., Muskegon, Michigan 49442).
(d) Micro condenser - 19/22 ₮; K-569250 (Kontes Glass Company, Vineland N.J. 08360).
(e) Concentrator tube - Mills type, 19/22 ₮ with stopper, 10 ml graduated in 0.1 ml up to 1.0 ml; K-570050 (Kontes Glass Company).
(f) Alkali dehydrochlorination reagent - Dissolve 2 g KOH in 100 ml ethanol.
(g) Ethanol - water, 1+1 - Combine equal parts by volume of distilled water and ethanol.
(h) Gas chromatograph - Equipped with electron capture detector and 6' x 4 mm id glass column containing either (1) 10% DC-200 or (2) 1:1 mixture of 15% QF-1 + 10% DC-200 on 80-100 mesh Chromosorb W(HP),

Operating conditions: N_2 flow 120 ml/min; temperatures, column and detector 200°C, injector 225°C; concentric design electron capture detector operated at DC voltage to cause 1/2 scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} amp.

Procedure

Accurately pipet, into a 10-ml Mills tube, 2 ml of a petroleum ether solution of sample extract (6% of 15% Florisil eluate (6)) containing concentrations of pesticides suitable for subsequent GLC analysis. Add 1 ml of 2% ethanolic KOH and a few carborundum chips and fit the tube with a micro condenser.

NOTE: Avoid getting alkali on the ground glass joint: light greasing of joint with silicone lubricant may prevent sticking.

With a test tube clamp, hold the tube over an opening in the steam bath in such manner that gentle boiling occurs. When the volume has been reduced to about 1 ml, insert the tube completely into the steam bath opening and heat vigorously for 15 minutes or until the volume reaches 0.2 ml. Remove tube from the steam. If a precipitate has formed, as is often the case with extracts containing fatty substances, add a few drops of 2% ethanolic KOH and warm gently in steam with swirling until the precipitate dissolves. After the solution has cooled slightly, add about 2 ml ethanol-H_2O (1 + 1). Allow solution to reach room temperature and pipet 1 or 2 ml hexane into tube. Stopper tube with ground glass, invert, shake vigorously for about 30 seconds, and allow solvent layer to separate sharply. With microliter syringe, carefully withdraw aliquot of opper layer for determination by GLC.

NOTE: Separation of phases should be sharp so that solution withdrawn for GLC analysis will be free from alkali.

DISCUSSION

Development of Method

Initial experimentation was performed to establish the reaction conditions which would give complete and rapid dehydrochlorination of p,p'-DDT, o,p'-DDT, o,p'-TDE, methoxychlor, and Perthane and which would permit complete recovery of the respective olefins. Work was done to determine the effect on olefin formation of fatty substances not removed during sample cleanup and the capacity of the reaction to eliminate fatty substances.

Several considerations found necessary for practical and reliable use of the alkali treatment have been incorporated into the method and are briefly discussed. The steam bath was chosen as a source of heat because of its ready availability and convenience. The Mills reaction tube was fitted with a micro condenser to eliminate losses due to volatilization, which often occurred in the absence of the condenser. Both KOH and NaOH have been used to the satisfaction of previous investigators. The more frequent use of KOH by other workers and its higher solubility in ethanol made it the choice for this work. An alkali concentration of 2% has been widely used, and was found ideal for treatment of aliquots of cleaned-up sample extracts containing quantities of bis(phenyl) chloroethane pesticides ranging from a few nanograms to 100 μg. In order to provide sufficient reflux time and temperature, it was necessary that the initial volume of ethanol be in excess of 0.5 ml. When smaller volumes were used, dehydrochlorination was usually incomplete. Emulsions often occurred during extraction of the olefin into hexane after saponification of fatty substances

The use of ehtanol + water (1+1) instead of water as the diluent resulted in a sharp separation of hexane and aqueous layers. Recoveries of olefins were not adversely affected if the ethanol content was less than about 70%. Less than 30% ethanol did not adequately enhance separation of the two layers. The non-volatile fatty substance transferred to acetonitrile by partitioning a petroleum ether solution of butterfat with acetonitrile was used in tests to evaluate the effects of fatty substances on the reaction. Experiments in which varying volumes of 2% ethanolic KOH were used to saponify 350 mg portions of this butterfat showed that each 1 ml of 2% KOH would saponify about 50 mg of the fat. When the weight of fatty substances exceeded about 50 mg, complete dehydrochlorination of Perthane (40 µg) and methoxychlor (4.0 µg) was not obtained. However, p,p'-DDT (8.0 µg) was completely dehydrochlorinated in the presence of 100-120 mg of fat. Additional experimentation showed that complete dehydrochlorination of methoxychlor and Perthane did not occur until the fat was completely saponified; these were the bis(phenyl) chloroethanes most resistent to dehydrochlorination. Quantities of Perthane and p,p'-DDT up to 100 µg in the presence of not more than 50 mg butterfat were readily dehydrochlorinated with 1 ml of 2% KOH at steam bath temperature; dehydrochlorination of larger amounts of pesticide was not attempted. Hexane, because of its higher boiling point and greater ease of drawing into a microsyringe, was used instead of petroleum ether, to extract the olefin after reaction in order to avoid possible errors in quantitation.

Effect on Selected Chemicals

The dehydrohalogenation reaction, as described under "Method", was applied to p,p'-DDT (0.8 µg), o,p'-DDT (0.8 µg), p,p'-TDE (2.0 µg), o,p'-TDE (0.4 µg), methoxychlor (4.0 µg), and Perthane (40 µg). Quantities shown in parentheses were chosen for ease in GLC determination. The pesticides were treated individually in petroleum ether, in 6% ethyl ether/petroleum ether Florisil eluates (6) containing the equivalent of 6 g of Kale, and in petroleum ether containing 30-60 mg fatty substances extracted from butter by partitioning (6) between petroleum ether and acetonitrile. Gas chromatography with electron capture detection, operated as described under "Method", was used for all determinations.

Each pesticide was completely altered in each of the solution types, i.e., none of the parent compound remained after treatment as described under "Method". Percent recoveries of the respective olefins were calculated according to the following equation:

$$\frac{\text{wt. olefin compound determined by GLC}}{\text{wt. parent compound represented in aliquot to GLC}} \times$$

$$\frac{\text{wt. parent compound/mol. wt}}{\text{wt. olefin compound/mol. wt.}} \times 100$$

Recoveries of olefins approximated 100% and ranged from 86% for p,p'-DDE and o,p'-TDE olefin in petroleum ether of 110% for p,p'-DDE in the extract from butter. Two olefin derivatives, the cis and trans isomers, are formed from o,p'-TDE (7). These have identical retention times on the two GLC columns used in this work and were quantitated as a single compound.

Several additional common organochlorine pesticides and polychlorinated biphenyls (PCB) were subjected to the described alkali treatment. All tests were made with petroleum ether solutions of the chemical under study. The quantity of each chemical was chosen for ease of determination by GLC and is given in parentheses.

Polychlorinated biphenyls ranging from 21 to 60% average chlorine content were stable to this treatment. Complete recoveries were obtained for the commercial PCB mixtures, Aroclors 1221 (16 μg), 1232 (16 μg), 1242 (16 μg), 1254 (8 μg), and 1260 (8 μg).

Recoveries of unchanged aldrin (0.4 μg), dieldrin (0.4 μg), and Endrin (0.4 μg) ranged from 70 to 90%. No alteration products were detected.

Heptachlor (0.4 μg) and heptachlor epoxide (0.4 μg) were markedly affected recoveries of the original compound ranged from 30 to 50%. Minor GLC peaks were observed on the 10% DC-200 column at retention times relative to aldrin of 1.63 after treatment of heptachlor epoxide and 0.59 and 0.93 after treatment of heptachlor.

The alkali treatment completely eliminated lindane (0.2 μg) and the alpha (0.2 μg), beta (0.2 μg), and delta (0.2 μg) isomers of BHC. Following the reaction, only small early eluting gas chromatographic peaks, presumably from trichlorobenzenes, were observed.

About 40% of mirex (4.0 μg) remained unchanged after reaction; sometimes a minor GLC peak appeared at a retention time relative to aldrin of 1.83 on the 10% DC-200 column.

Endosulfan I and II, treated separately, were completely eliminated. Each isomer gave a single alteration product with retention time relative to aldrin of 1.82 on the 10% DC-200 column and 2.23 on the 1:1 10% DC-200/15% QF-1 column. The peak height of the alteration product was approximately one-tenth the peak height of the parent compound. A structure for this derivative has been proposed (8).

Endosulfan sulfate also was completely eliminated. Two alteration products were obtained with retention times relative to aldrin of 0.28 and 0.38 on the 10% DC-200 column.

Dicofol (1.0 μg) was completely eliminated, but only 65% of the major alteration product, 4,4'-dichlorobenzophenone, was recovered. A minor peak was observed in the chromatogram at a retention time relative to aldrin of 1.71 on the 10% DC-200 column. The 4,4'-dichlorobensophenone (2.0 μg) was not affected by treatment with alkali.

The products resulting from alkali treatment of toxaphene (10.0 μg) gave a multicomponent chromatogram but consisting of components with earlier retention times than toxaphene itself.

The electron capture GLC responses to sulfur (20 μg), frequently encountered in residue analysis at retention times relative to aldrin of 0.23, 0.55, and 1.13 on the 10% DC-200 column, were eliminated by the alkali treatment.

Application of Method

The most obvious use of the alkali treatment is to form the olefins of bis(phenyl) chloroethane pesticides for confirmation of residue identity. The complete yield and recovery of the olefin derivative makes possible quantitative confirmation of a residue of the parent pesticide. For example, a residue of p,p'-DDT can be quantitated before alkali treatment and verified by quantitation as p,p'-DDE after treatment.

In addition, the GLC retention time region of the reacted compound can be examined for presence of peaks from unreacted and presumably interfering substances. We have found the alkali treatment especially useful in connection with the real or suspected presence of residues of PCB. In this case the characteristic olefin derivatives of p,p'-DDT, o,p'-DDT, and p,p'-TDE can be formed and the GLC retention time region underlying the parent compounds can be examined. The stability of PCB to alkali, with no change in the GLC pattern, is a characteristic which can be readily utilized in confirmation of the identity of this complex residue. The high recovery of unreacted dieldrin, endrin, and aldrin following alkali treatment likewise can be of value in the confirmation of identity of these pesticides.

Cleaned-up extracts of some samples may contain non-pesticidal substances which give rise to electron capture response. Other extracts may contain fatty substances, not removed by the cleanup, which can prohibit application of some tests, e.g., thin layer chromatography. This is particularly true of the 15% ethyl ether/petroleum ether Florisil column elutate (6) for non-fatty samples such as carrots and fatty samples such as some fish. Electron capturing substances present in carrots must be eliminated before determination of dieldrin and/or endrin residues. Treatment with alkali serves well for this purpose. Extracts of fatty samples may require treatment to eliminate both electron capturing substances and non-volatile fatty substances. In many instances, thin layer chromatography or microcoulometric GLC cannot be accomplished prior to alkali treatment. Electron capture responses to sulfur, often a source of annoyance to the residue chemist, are also eliminated by this treatment.

PERCHLORINATION OF PCBs

Subsection 9,B,(2), IX describes a method for confirmation of polychlorinated biphenyls (PCBs) based on perchlorination of all compounds to yield a single derivative, decachlorobiphenyl, followed by electron capture gas chromatography. Although the method was developed for mother's milk and is described in detail only for this substrate, perchlorination undoubtedly has much wider applicability to other types of samples. Suitability must be verified in each particular situation, however, by recovery studies on fortified check samples.

INFRARED SPECTROSCOPY

I. INTRODUCTION:

Infrared spectroscopy is the most powerful single technique available for the identification of organic compounds and is almost without equal as empirical proof of identity. The disadvantages of infrared are low sensitivity, requirement of a relatively pure sample, and the training and experience necessary to interpret spectra. The low sensitivity requires that gram quantities of sample be processed. The requirement of a relatively pure sample dictates the use of a stringent sample cleanup procedure plus additional cleanup of the extract either by gas chromatographic separation or thin -layer chromatography.

Infrared is sensitive to at least 1 μg and has been utilized at the 0.1 μg level. Thus, it is considerably less sensitive than either gas or thin layer chromatography.

A number of techniques have been developed for infrared; however, the technique presented here, potassium bromide pellets, is the most sensitive and dependable. Considerable experience is necessary in the preparation of pellets to minimize contamination, which is the principal problem inherent in this technique.

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9. Perkin-Elmer Corp., Operating Manual, I.R., 337.

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II. EQUIPMENT:

A. Perkin-Elmer IR-337, or equivalent, equipped with a 6X beam condenser and holder for 1.5 mm KBr discs.

B. Micro KBr equipment, Perkin-Elmer ultra micro dye assembly.

C. Press, capable of exerting 200 pounds of pressure.

D. Micro mortar and pestle.

E. Fine tipped forceps.

F. 50 µl syringe

G. White glove liners.

H. Vacuum pump.

I. Oven at 60-70°C

J. Micro Spatula.

K. Thin-layer equipment.

L. Stream splitters: 1-100 and 1:1000.

M. Capillary Tubing, borosilicate glass, 2mm ID

N. Pipe cleaners, dyed to various colors.

O. Desiccator, vacuum

P. Evaporator, vacuum, rotary.

III. REAGENTS:

A. Standard pesticides

B. Hexane, reagent, redistilled*

C. Potassium bromide, infrared grade.

D. Aluminum oxide G.

E. Ethyl ether, anhydrous, reagent grade.

F. Ethanol, 95%

G. Rhodamine B dye.

H. Silica gel G.

I. Acetone, reagent, redistilled*.

J. Methanol, reagent.

K. Chloroform, reagent.

L. Buffer, pH 6.0.

M. Methylene chloride, reagent grade, redistilled.*

N. Palladium chloride.

O. Hydrochloric acid.

P. Potassium bromide wick-sticks, Harshaw Scientific Co.

IV. INSTRUMENT CALIBRATION:

A. Gain Adjustment

1. Decrease gain until there is a sluggish response when the sample beam is blocked and unblocked.

2. Increase gain until the correct response is obtained.

   a. Partially block the sample beam with your thumb to obtain 10% downscale deflection.

   b. Rapidly, remove your thumb and note the overshoot.

   c. Adjust until you obtain 1-2% overshoot.

B. Balance Adjustment

1. Partially block the sample beam with your thumb.

2. Change balance control to bring the pen to about 50%

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*Redistilled in all-glass apparatus.

3. Simultaneously block both beams and adjust control to no downscale or upscale drift.

4. Slight upscale drift can be tolerated, however, downscale cannot.

C. Zero Adjustment

1. Partially block the sample beam until the pen reads about 5%.

2. Very slowly continue blocking the beam until it is completely blocked.

3. If pen does not read zero:

    a. Remove pen tower cover.

    b. Loosen the screw holding the pen carriage on the slide wire.

    c. Set pen to zero.

    d. Tighten screw.

4. Repeat procedure (1 through 3) until zero is properly adjusted.

D. 100% adjustment

1. Be sure both beams are not blocked

2. With the 100% adjustment, set the pen to 99-100%.

E. General

1. Always make the adjustments in the order in which they have been presented.

2. Always check these adjustments before sample analysis.

3. Remember that a change in the zero adjustment will necessitate a change in the 100% adjustment.

V. SAMPLE PREPARATION:

A. Use enough sample to provide a sufficiently large concentration of the compounds under analysis to allow infrared observation.

B. Use a cleanup procedure which will provide relatively pure pesticide compounds.

1. Trapping of gas chromatograph effluent-capillary procedure.

    a. Attach splitter to the inlet to the EC detector (split ratio, 1/100-1000).

    b. Collect effluent from splitter on KBr in cooled tube, prepared as follows:

        (1) Use a 3" length of capillary tubing (2mm I.D.).

        (2) Place a pipe cleaner in the tube as a reagent support.

        (3) Pack the tube with about 10 mg of dry KBr.

        (4) Hold the packed tube at 150°C.

        (5) Just prior to use, cool the tube in a dessicator just below room temperature.

    c. Collect center fraction of peak desired, by providing intimate contact between packed capillary and outlet of splitter arrangement.

    d. Force KBr out of tube into micro dye, using pipe cleaner.

    e. Prepare KBr pellet.

2. Wick-Stick trapping procedure

    a. Collect desired peak by holding wick-stick to exit of splitter.

    b. Concentrate pesticide at tip of wick-stick by procedure described in the wick-stick kit.

    c. Break off tip of stick and prepare pellet therefrom.

3. Thin-layer cleanup and separation

    a. Chlorinates pesticides

        (1) Prepare plates using the mechanics presented under "Thin-layer chromatography."

            (a) Aluminum oxide G.

            (b) Activate in an oven at 155°C for 2.5 hours.

            (c) Store over Drierite until used.

(2) Concentrate extract to 1-0.1 ml, depending on concentration of agent in the extract.

(3) Spot a sufficient amount of the extract, and the appropriate standards, on the plate.

(4) Develop chromatograms with 1% ethyl ether in hexane.

(5) Spray lightly with 0.01% rhodamine B in 95% ethanol.

(6) Remove each spot desired, by vacuum, using a glass medicine dropper plugged with glass wool.

(7) Elute the pesticide from the adsorbent with 5 ml of 4:1 hexane-ethyl ether mixture.

(8) Concentrate the eluate to about 0.1 ml.

(9) Mix sample and KBr.

    (a) Weigh out 7 mg dry infrared quality KBr into a warm micro-mortar and lightly tamp into a small cake.

    (b) Add concentrated eluate to the KBr to 2 μl increments, allowing time for solvent evaporation between each addition. Put eluate on KBr, not the mortar.

b. Organothiophosophate pesticides.

(1) Prepare plates using the mechanics presented under "Thin-layer chromatography."

    (a) Mix 30 g silica gel G with pH 6 buffer in a 250 ml Erlenmeyer flask.

    (b) Shake vigorously for 1 minute.

    (c) Apply as a 250 micron layer.

    (d) Let plates air dry overnight.

    (e) Wash plates twice by letting acetone migrate up plates for 20 cm.

    (f) Air dry.

(2) Concentrate sample to 0.3 ml in methylene chloride.

(3) Using a micropipette, apply sample to plate, in increments, drying between applications.

(4) Develop with 1.75% methanol in chloroform.

(5) Air dry.

(6) Spray the plate with 5 ml of a 5% solution of palladium chloride and 1 ml of HCL, diluted to 100 ml in 95% ethanol.

(7) Allow plate to dry for 30 minutes at room temperature (may be necessary to hold plate overnight before all thiophosphates are discernible).

(8) Locate spots of interest and remove each by the method given for chlorinated pesticides.

(9) Extract the adsorbent with five 1 ml portions of hot acetone into a 25 ml microflask.

(10) Evaporate to dryness under vacuum in a rotating evaporator.

(11) Add 1 ml of CCl_4, rinse the walls of the flask and re-evaporate.

(12) Take up the residue in CCl_4 and concentrate to about 0.1 ml

(13) Place 5-7 mg of dry infrared KBr in a warm mortar and add the concentrated residue as instructed for chlorinated pesticides.

VI. ALTERNATE METHODS OF MIXING CONCENTRATED SAMPLE EXTRACT AND KBR:

A. Wick-stick procedure

1. Place concentrated extract in vial of wick-stick kit.

2. Place wick-stick in vial and allow solvent to evaporate, concentrating the pesticide on the tip of the stick.

3. Break off tip and use to make pellet.

B. New procedure of Blinn

1. Prepare 13 mm pellet without pesticides.

2. Using this pellet as a micro-mortar, put lightly heated KBr powder thereon and add solution dropwise.

    3. Remove loose KBr into micro-dye and scrape the surface of the 13 mm pellet into the micro-dye.

    4. Prepare 1.5 mm micro-pellet.

  C. Syringe procedure

    1. Place sample extract into a syringe equipped with a discharge controller.

    2. Eject a drop of the solution to the syringe tip, dip into KBr powder and suspend on the needle.

    3. Continue ejecting solution onto KBr, drying between injections.

    4. Place KBr in micro-dye and prepare pellet.

VII. PREPARATION OF KBR PELLET:

A. Transfer the KBr sample to the micro-dye.

B. Assemble the dye.

C. Assure that the sample is spread evenly by rotating the top ram under slight hand pressure.

D. Press and evacuate the dye.

E. Remove the pellet and analyze.

F. Clean dye immediately after use.

VIII. ANALYSIS AND INTERPRETATION:

A. Turn on instrument.

B. Place pellet in holder on instrument.

C. Recheck

  1. Gain

  2. Balance

  3. Zero

  4. 100% adjustment

D. place paper on drum.

E. With scan control in stop, place range switch in proper range to correspond with chart paper.

F. Scan sample.

G. Turn scan switch to stop.

H. Change range switch.

I. Change chart paper.

J. Replace drum in well.

K. Turn scan control to "reset."

L. Turn drum to beginning of range.

M. Put pen on paper.

N. Scan second range.

O. Interpret spectrum by comparison with spectra from standard pesticides.

IX. MISCELLANEOUS NOTES:

A. Do not turn instrument on and off during the day it is being used.

B. Do not leave instrument stand with pen above 100%.

C. Set gain, balance, and 100% in that order and with sample in beam. Check gain at about 4.5 microns.

D. Turn scan control to stop before changing range switch.

E. Eliminate all possible contamination when making micro KBr pellets.

F. When using thin-layer cleanup, remember "Thin-layer, Notes on Procedure."

G. When trapping from gas chromatograph:

1. Minimize contamination from substrate, column, and previous instrument use.
2. Work with as much sample as can be collected by taking a center cut of the peak.

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U. S. Food & Drug ADMINISTRATION (Revision 7/1/70).

POLAROGRAPHY

640.1 Introduction. The development of oscillopolarographic instrumentation and techniques has caused a renewed interest in the use of polarography for residue determination. This technique is rapid and specific. Its sensitivity is comparable to colorimetry.

640.2 Recommended Literature References

(1) Gajan, R. J., Residue Reviews, Vol. 6, pp 75-86, and Vol. 5, pp 80-99, Springer Verlag, New York, 1964.

Gajan discusses the applications of polarography for the detection and determination of pesticides and their residues. He shows 12 single sweep polarograms with comparison of derivative and regular wave, result of degradation, and nitro derivatives. He lists 28 references.

(2) Martens, P. H., and Nangniot, P., Residue Reviews, Vol. 2, pp 26-50, Springer Verlag, New York, 1963.

Martens and Nangiot review polarographic applications for determining: copper, mercury, arsenic, tin and sulfur compounds; natural organic products such as nicotine, rotenone, and pyrethins; and synthetic organic compounds. They list 163 references.

(3) Gajan, R. J., JAOAC 48, 1028-1037 (1965).

Gajan discusses the practical application of polarographic techniques to the determination of pesticide residues. He lists 46 references.

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POLAROGRAPHIC PROCEDURE FOR PESTICIDE RESIDUES

641.01 References. Official Methods of Analysis of the Association of Official Analytical Chemists 11th Edition, Sections 29.034-29.038. Included are official AOAC methods for parathion, methyl parathion, diazinon, and malathion. These methods are indicated by (AOAC) at the respective numbered paragraphs. Paragraphs describing methods not covered by the AOAC methods include in reference to the basic literature.

Study leading to AOAC official status:
Gajan, R. J., JAOAC 52, 811-817 (1969).

641.02 Application. This procedure will enable the residue analyst to check results obtained with various multiple residue systems, using a portion of the same cleaned up sample extract used for multiple residue determination.

641.1 Apparatus

Cathode-ray Polarotrace K1000 or
Davis Differential Cathode-ray Polarotrace A1660
Silver wire electrode. A No. 20 or N. 22 silver wire on which a very thin coating of silver chloride has been deposited.
Polarographic cells (at least 6)
Capillaries (at least 3 extra)
Stop watch

641.2 Reagents

Acetic acid. Glacial, ACS grade
Acetone. Redistilled 56.5°C. Add 1 g $KMnO_4$ per 4 L acetone being distilled
Alcohol. 95% USP
Ethyl acetate. Reagent grade, redistilled, 77°C ± 1°C
Hydrochloric acid. 37-38%, ACS grade
Lithium chloride
Mercury. Purified
Methanol
Nitrogen. Prepurified, water pumped.
Potassium chloride. ACS grade
Potassium hydroxide. ACS grade
Potassium permanganate. ACS grade
Sodium acetate. ($NaOAc \cdot 3H_2O$). ACS grade
Sodium chloride. ACS grade
Sodium nitrite. ACS grade
Tetramethylammonium bromide. Eastman white label No. 670
Standard pesticide solutions. Prepare standard solutions containing 1 mg of pesticide per ml of ethyl acetate; store at 0°C.

641.3 General Method. Transfer suitable aliquot (1.0 ml) of cleaned up extract to 50 ml erlenmeyer flask and evaporate just to dryness under gentle jet of dry air at room temperature. Extracts must be in peroxide-free solvents.

Dissolve this residue in a definite volume of solvent as directed by specific procedures (641.42) for the various pesticides and add the required amount of supporting electrolyte, mix well, and transfer 5.0 ml or less of the mixture to a polarographic cell. (Since good polarotraces may be obtained using only 0.5 ml of solution in a polarographic cell a minimum of 0.25 ml solvent could be used to dissolve residue.) Bubble nitrogen through cell solution for 5 min and polarograph at 25°C ± 1°C. over designated voltage scan.

Measure any waves appearing within ±0.10 volt of peak potential of pesticide being determined. Peak potential of pesticide being polarographed is determined by polarographing a standard of the pesticide dissolved in the same solvent and electrolyte as the sample, immediately before or after sample. The standard solution should contain approximately the same amount of pesticide as the sample. When uncleaned solutions are polarographed the peak potential may shift slightly due to density of the cell solution.

Estimate amount of pesticide in the solution by comparing wave height of sample solution with that of standard solution.

A valuable check on qualitative determination of the pesticide, if in doubt, is the standard addition technique, i.e., add a known amount of pesticide standard solution to cell containing the sample solution and note any increase in wave height. The peak potential of the standard pesticide should be the same as that of the pesticide in the sample if they are the same compound. The amount of total pesticide in the cell can be calculated after correcting for volume change.

641.4 Application to Specific Residues

641.41 Suitability for Mixtures. By the judicious choice of supporting electrolyte one can determine any admixture of the pesticides in 641.42 in approximately 20 minutes. Polarographic interference between compounds noted in 641.42 are sometimes avoided by the separations effected in the extraction and cleanup procedures. The analyst should be aware of which compounds may possibly be present in a sample solution.

The extraction and cleanup procedures now being used for the various multiple detection procedures are adequate for the polarographic procedures described here provided the same precautions as to purity of reagents and solvents are maintained.

641.42 Specific Residues

641.42a (AOAC) Parathion and/or methyl parathion

Limitations. Limit of quantitative detection is 0.01 ppm based on 1 g crop sample in 1 ml cell solution.

Parathion, methyl parathion, and paraoxon give similar polarographic responses. Therefore, report all results as total of the three unless the specific analog has previously been identified or unless the prior analytical method permits only certain of the compounds to be in the sample solution.

Electrolyte solution. Dissolve 2.72 g $NaOAc\cdot 3H_2O$ and 1.17 g NaCl in 100 ml redistilled H_2O and adjust pH to 4.8 with glacial HOAc.

Polarographic determination. Dissolve residue from evaporation in a definite volume of acetone and add an equal volume of electrolyte solution. Proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less of the mixture to a polarographic cell..."

The peak potential for parathion is -0.68 ± 0.05 volt vs. mercury pool reference electrode and -0.70 ± 0.05 volt vs. silver wire reference electrode.

Prepare working standard solutions by diluting appropriate amounts of stock solution with acetone.

641.42b Guthion

Reference. Bates, J. A. R., Analyst 87, 786-790 (1962).

Limitations. Limit of quantitative detection is 0.01 ppm based on 1 g crop sample in 1 ml cell solution.

Guthion and its oxygen analog give similar polarographic responses. Therefore, report all results as total of the two unless the specific analog has been previously identified.

Electrolyte solution. Prepare aqueous solution which is 0.5M HOAc and 0.2M KCl.

Polarographic determination. Dissolve residue from evaporation in a definite volume of acetone and add an equal volume of electrolyte solution. Proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less to a polarographic cell..."

The peak potential for Guthion is -0.70 ± 0.05 volt vs. either mercury pool reference electrode or silver wire reference electrode.

641.42c (AOAC) Diazinon

Limitations. Limit of quantitative detection is 0.2 ppm based on 1 g crop sample in 1 ml cell solution.

Diazinon and its oxygen analog give similar polarographic responses. Therefore, report all results as total of the two unless the specific analog has been previously identified.

Electrolyte solution. Dissolve 7.7 g tetramethylammonium bromide in 300 ml H_2O (0.1M). Add 115 ml HOAc and dilute to 500 ml with H_2O.

Polarographic determination. Dissolve residue from evaporation in a suitable amount of electrolyte solution and proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less to a polarographic cell..."

The peak potential of diazinon is -0.90 ± 0.05 volt vs. either mercury pool reference electrode or silver wire reference electrode.

Prepare working standard solutions by diluting appropriate amounts of stock solution with petroleum ether. Evaporate carefully to dryness and proceed as with sample determination.

641.42d (AOAC) Malathion

Limitations. Limit of quantitative detection is 0.3 ppm based on 1 g crop sample in 1 ml cell solution.

Malathion and its oxygen analog give similar polarographic responses. Therefore, report all results as total of the two unless the specific analog has been previously identified.

Electrolyte solution. Dissolve 15.4 g tetramethylammonium bromide in 300 ml H_2O (0.2M). Add 0.2 g lithium chloride and 4.1 ml concentrated HCl, and dilute to 500 ml with H_2O.

Polarographic determination. Dissolve residue from evaporation in a definite volume of methanol. Add 1/2 as much 0.1N KOH. Let stand for 3 min and add an amount of electrolyte solution equal to the amount of methanol used. Let stand 5 min. Proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less to a polarographic cell..."

The peak potential for malathion is -0.85 ± 0.05 volt vs. a mercury pool electrode and -0.82 ± 0.05 volt vs. a silver wire electrode.

Prepare a working standard solutions by diluting appropriate amounts of stock solution with methanol.

Notes. Diazinon interferes with malathion since reduction is at the same peak potential. However, malathion does not interfere with diazinon in procedure 641.42c. If diazinon is suspected check for diazinon according to 641.42c. If diazinon is found present, the amount of malathion in the sample can be estimated with an accuracy of ±10% by subtracting the amount of diazinon found by procedure 641.42c from the total amount of pesticide found by procedure 641.42d when calculated as malathion. The same amounts of diazinon and malathion give approximately the same polarographic wave heights when polarographed using the electrolyte system described in 641.42d.

641.42e Dimethoate

Reference. Gajan, R. J., and Gaither, R. A., unpublished method.

Limitations. Limit of quantitative detection is 0.05 ppm based on 1 g crop sample in 1 ml cell solution.

Electrolyte solution. 0.1 N KOH in H_2O.

Polarographic determination. Dissolve residue from evaporation in a definite volume of ethanol and add the required amount of electrolyte to maintain a ratio of 3 parts electrolyte to 2 parts ethanol. Proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less of the mixture to a polarographic cell..."

The peak potential of dimethoate is -0.30 ± 0.05 volt vs. mercury pool reference electrode and -0.55 ± 0.05 volt vs. silver reference electrode.

641.42f Carbophenothion

Reference. Nangnoit, P., Anal. Chem. Acta. 31, 166-174 (1964).

Limitations. Limit of quantitative detection is 0.2 ppm based on 1 g crop sample in 1 ml cell solution.

Electrolyte solution. 50% w/v KOH in H_2O.

Polarographic determination. Dissolve residue from evaporation in a definite volume of ethanol. Add an equal volume of electrolyte, mix well and proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less of the mixture to a polarographic cell..."

The peak potential for carbophenothion is 0.28 ± 0.05 volt vs. a mercury pool reference electrode and -0.43 ± 0.05 volt vs. silver wire reference electrode.

641.42g Carbaryl.

Reference, Gajan, R. J., Benson, W. R., and Finocchiaro, J. M., JAOAC 48, 958-962 (1965).

Limitations. Limit of quantitative detection is 0.05 ppm based on 1 g crop sample in 1 ml of cell solution.

Electrolyte solution. Mixture of glacial HOAc, 1.0 N $NaNO_2$ in H_2O, and 50% w/v KOH in H_2O, in ratio of 1:1:3.

Polarographic determination. Dissolve residue from evaporation in a definite volume of glacial acetic acid. Add an equal volume of 1.0 N NaNO and let stand for 3 min. Add an amount of 50% KOH equal to three times the volume of acetic acid used. Let stand for 15 min and proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less of the mixture to a polarographic cell..."

The peak potential for carbaryl is -0.45 ± 0.05 volt vs. mercury pool reference electrode and -0.68 ± 0.05 volt vs. a silver wire reference electrode.

641.42h DDT in the Presence of Toxaphene (100X)

Reference, Gajan, R. J., and Link, J., JAOAC 47, 1119-1124 (1964).

Limitation. Limit of detection is 0.5 ppm based on 1 g crop sample in 1 ml cell solution.

Electrolyte solution. Dissolve 7.703 g tetramethylammonium bromide in 250 ml distilled H_2O (0.2M).

Polarographic determination. Dissolve residue from evaporation in a definite volume of acetone and add 1.5 times as much ethanol. Add a volume of electrolyte equal to that of the mixed solvent. Proceed as directed in the general method, 641.3, starting with "...transfer 5.0 ml or less of the mixture to a polarographic cell..."

The peak potential for DDT is -0.60 ± 0.05 volt vs. mercury pool reference electrode and -0.70 ± 0.05 volt vs. silver wire reference electrode.

Notes. Parathion interferes with DDT in this procedure; however, the two can be separated by Florisil column chromatography. DDT is found in the 6% Florisil column eluate; parathion in the 15% eluate (see 201). Other analogs of DDT containing the trichloroethane configuration will also interfere; however, they can also be separated from parathion by Florisil column chromatography. Their absence should be checked for by GLC or TLC.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

See Subsection 6N through 6S of the EPA Pesticide AQC Manual for a discussion of high performance liquid chromatography (HPLC) including instrumentation, theory and principles, columns and solvents, practical aspects of successful operation, and applications to pesticide analysis.

By far the most popular mode for the determination of pesticide residues has been reverse phase HPLC on chemically bonded C_{18} columns combined with ultraviolet adsorption detection. However, the electrochemical LC detector has great promise for residue analysis because of its subnanogram sensitivity for certain compounds (see Determination of Halogenated Anilines and Related Compounds by HPLC with Electrochemical and Ultraviolet Detection, Lores, E. M., Bristol, D. W., and Moseman, R. F., J. Chromatogr. Sci., 16, 358 (1978).

Retention and response data for pesticide standards and detailed analytical procedures for residues will appear in future revisions of this section as they are developed and tested in EPA laboratories. HPLC data for 166 pesticidal compounds have been compiled by J. F. Lawrence and D. Turton in J. Chromatogr., 159, 207 (1978). This reference lists the column packing, column dimensions, mobile phase, elution volume, nature of the pesticide (standard or residue from a particular substrate), UV detection wavelength, and the literature reference.

GENERAL COMMENTS FOR THE MAINTENANCE AND REPAIR OF INSTRUMENTS

The subject of instrumental servicing is obviously far too complex to treat in a meaningful way in a manual such as this. In fact, a full treatment would undoubtedly require an entire manual the size of this one.

The few comments which are offered here are primarily intended for those laboratories which are a part of the U.S. Environmental Protection Agency or which have formal contractual agreements with EPA and are therefore eligible to obtain full benefits from the electronic repair facility.

The Instrument Shop at Research Triangle Park, N.C. is fully equipped to handle all repairs, modifications and calibrations on the Tracor MT-220 or MT-222 gas chromatographs and on miscellaneous brands of strip chart recorders. The services of the shop are available to all EPA regional laboratories and to the Epidemiologic Studies and Human Monitoring laboratories holding contracts with EPA, these services to be supplied on a cost-free basis. In such instances where the service required is covered by a no-cost manufacturer's warranty, the manufacturer or distirbutor should be contacted for repairs.

The following services are available to the qualified laboratories:

1. Repair of Electron Capture Detectors including foil replacement, rods, BNC connectors, Teflon internal parts, and other parts as required.
2. Repairs, calibrations, modifications to: Electrometers, Programmers, Temp-Set Controllers, E C Power Supplies, Recorders, Microcoulometer and FPD Detector Systems.

In addition to the items above, a variety of miscellaneous repairs are performed on blower motors, limit switches, oven heaters, thermometers, etc., on a "one-for-one" basis.

Replacement modules, components, or recorders used with the MicroTek GC MT-220 are available from the Instrument Shop

These include in part:

Electrometers - complete or integral components.

Programmers - complete or integral components.

Temp. Set controllers - complete or integral components.

Recorders - Westronics or Honeywell - complete or integral components.

Flow Controllers, rotometers, damper systems, blower systems, heaters 50 or 100 watt, limit switches adjustable and preset oven coils, specified wiring, voltage monitor kits (for 110 V., A.C., only), accessory variacs, light duty Sola regulators, thermometers, signal cables (BNC-BNC), pilot lamps, multidials, 10 turn potentiometers, compensator PCB, Insulation (Microfibre).

Dohrman system components, power supplies, printed circuit boards (PCB's) and componets thereof.

A few items - Freon, Snoop, "O" rings, Septums, etc., should be purchased by the user and only requested on an emergency basis where the possibility of "down time" exists before normal purchase procedures can be accomplished.

Our services may be obtained by phone or mail, depending on the urgency. If "down time" is anticipated, a phone call should be made requesting the desired service or equipment. It is suggested that complete information be written prior to a telephone service call - model numbers, age of unit to be replaced if known, and pertinent data on the isolation service procedures already tried. It is imperative that one does not attempt to use the "Mobile Reserve" to "Stock" their laboratory. It is requested that all malfunctioning units be sent to the Instrument Shop for repairs or survey.

In case of instrument breakdown requiring on-site servicing, the appropriate area project coordinator should be contacted for discussion of the need/cost involved.

Problems encountered with the gas chromatograph and not definitely isolated as being electronic in nature should be channeled through Dr. E. O. Oswald, Chief, Chemistry Section, or discussed directly with J. F. Thompson, Analytical Quality Assurance Chief, both at RTP.

Detailed trouble shooting instructions for all instrument modules are far too lengthy for inclusion in this manual. Copies of such instructions may be obtained from the RTP Instrument Shop.

Materials to be shipped to the Instrument Laboratory for service should be placed in appropriate containers with sufficient packing to insure against damage. Articles should not be shipped COLLECT except when specific agreement has been reached to do so. We suggest the use of second-class air mail when possible and that insurance in the full amount of the units cost be purchased. Special shipping cartons and packing materials are used to send various items - "These are not to be discarded". The materials are expensive and should be returned or reused for future shipments to the Instrument Shop.

Phone: Mr. Frank Wilinski, 919-549-8411, X2508 (commercial) or 629-2508 (FTS).

Mail, Truck or REA Shipments:
Instrument Shop
Quality Assurance Section
Environmental Toxicology Division
EPA, Health Effects Research Laboratory
Room 113, Monsanto Bldg. (MD-69)
Research Triangle Park, NC 27711

ANALYTICAL QUALITY CONTROL

The term "quality control (QC)" may connote to some a system of contolling the quality of a manufactured product. The phrase is wisely applied in this role, but it also can be aptly used to indicate a "level of performance". It is in the latter sense that the term is properly applied in the chemical analysis for pesticide residues. A program of quality control is a means of assuring the output of reliable and valid analytical data.

A Systematic program of quality control is of equal importance in an analytical laboratory to any other activity performed by the laboratory. Notwithstanding, many laboratory administrators fail to recognize its importance, and they make no provision in time and resources for its incorporation in the overall laboratory program. The comment has often been heard from individual bench chemists to the effect that "we couldn't possibly fit the type of QC program recommended into our work schedule." Unquestionably, this statement has been a true one and has resulted from failure by the administrator to recognize and provide for quality control in the analytical program planning.

The unfortunate consequence of a lack of a systematic QC program is the output of highly questionable analytical data of little or no value for decision making. In the case of a regulatory laboratory, such data can not be introduced as evidence in a court because of the danger of its discreditation by the opposition. In a monitoring situation, such questionable data could, for example, lead to false conclusions as to the pesticidal profile of some sector of the environment.

In the preceding pages of this Manual, a number of multiresidue and specific residue analytical procedures have been presented. A number of these have been subjected to collaborative studies and are known to yield acceptable interlaboratory precision and accuracy. Yet, no method presented should be expected to produce unquestionable data unless it is conducted within the framework of systematic controls. Pesticide residue procedures in general are highly complex and exacting, requiring highly sophisticated electronic instrumentation. The lack of adequate controls is tantamount to a ship without a compass.

In 1976 a complete and separate manual for analytical quality control was developed, and it is now available in its 1st revised edition (1979). The specifics of a QC program for pesticide analysis area treated in this Manual. An outline of the EPA QC Manual is shown on the following page:

Title: Manual of Analytical Quality Control for Pesticides and Related Compounds in Human and Environmental Media

Author: Dr. Joseph Sherma, Department of Chemistry, Lafayette College, Easton, PA 18042

Revisions by: Dr. Joseph Sherma and Dr. Morton Beroza, The Association of Official Analytical Chemists, Arlington, Virginia.

Editors: Randall R. Watts and Jack F. Thompson, EPA, Research Triangle Park, NC

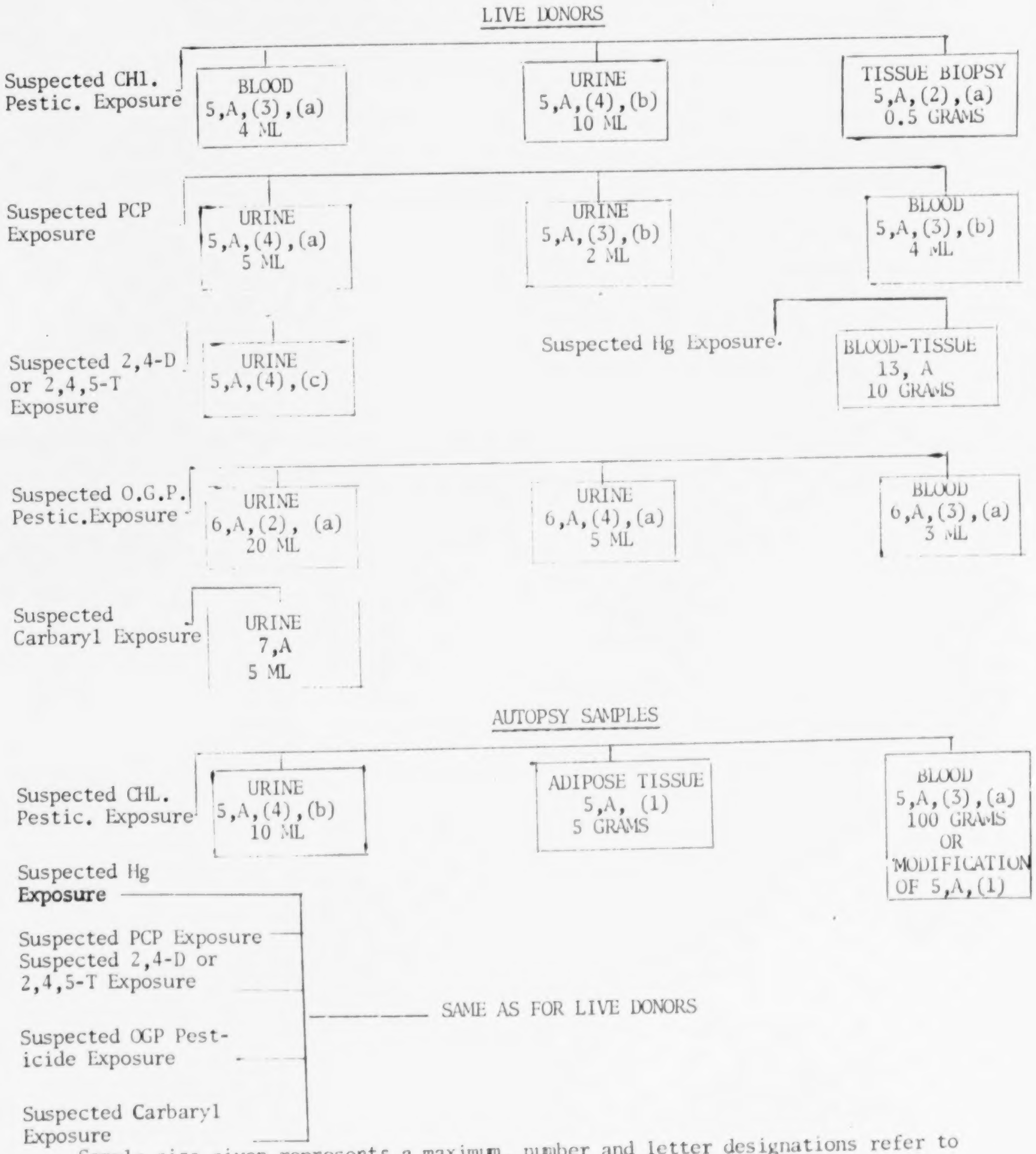
1. General Description of Pesticide Residue Analytical Methods.
2. Interlaboratory Quality Control.
3. Intralaboratory Quality Control.
4. Evaluation, Standardization, and Use of Materials for Pesticide Residue Analysis.
5. Operation of the Gas Chromatograph.
6. Additional Procedures in Pesticide Analysis.
7. Multiresidue Extraction and Isolation Procedures for Pesticides and Metabolites.
8. Confirmatory Procedures.
9. Maintenance, Troubleshooting, and Calibration of Instruments.
10. Training of Pesticide Analytical Chemists.

Persons wishing a copy of the QC Manual may write to the following address:

Environmental Toxicology Division
EPA, HERL (MD-69)
Research Triangle Park, NC 27711

1/4/71

TENTATIVE TISSUE, EXCRETA AND METHOD SELECTION FOR ABNORMAL PESTICIDE EXPOSURE CASES; BLOCK DIAGRAM


LIVE DONORS
Suspected CHl. Pestic. Exposure
BLOOD 5,A,(3),(a) 4 ML
URINE 5,A,(4),(b) 10 ML
TISSUE BIOPSY 5,A,(2),(a) 0.5 GRAMS
Suspected PCP Exposure
URINE 5,A,(4),(a) 5 ML
URINE 5,A,(3),(b) 2 ML
BLOOD 5,A,(3),(b) 4 ML
Suspected 2,4-D or 2,4,5-T Exposure
URINE 5,A,(4),(c)
Suspected Hg Exposure
BLOOD-TISSUE 13, A 10 GRAMS
Suspected O.G.P. Pestic.Exposure
URINE 6,A,(2), (a) 20 ML
URINE 6,A,(4),(a) 5 ML
BLOOD 6,A,(3),(a) 3 ML
Suspected Carbaryl Exposure
URINE 7,A 5 ML
AUTOPSY SAMPLES
Suspected CHL. Pestic. Exposure
URINE 5,A,(4),(b) 10 ML
ADIPOSE TISSUE 5,A, (1) 5 GRAMS
BLOOD 5,A,(3),(a) 100 GRAMS OR MODIFICATION OF 5,A,(1)
Suspected Hg Exposure
Suspected PCP Exposure
Suspected 2,4-D or 2,4,5-T Exposure
Suspected OGP Pesticide Exposure
Suspected Carbaryl Exposure
SAME AS FOR LIVE DONORS

Sample size given represents a maximum, number and letter designations refer to method number as listed in the TABLE OF CONTENTS.

PESTICIDE ANALYTICAL REFERENCE STANDARDS

The pesticide repository of the Environmental Toxicology Division was initially established at the former Perrine, Florida, location. The repository was created primarily to provide pesticide reference standards for Pesticide Community Studies and other field laboratories under contract to the United States Government to conduct pesticide monitoring programs. It was created in the belief that a central source of analytical-grade reference compounds would greatly assist in the assurance of accurate, reliable analytical data.

In addition to meeting its primary responsibility to its program laboratories, the ETD has extended this service to other nonprofit government and university laboratories on a discretionary basis as time and resources permit. Because of great demand from many sources, and limited supplies, the amount of each standard sent out is restricted to no more than 100 milligrams and the number of standards to only those necessary for limited immediate needs. The short shelf life of many standards is one of the reasons for restricting field pesticide inventories.

Most of the high-purity analytical-standard compounds carried in the repository stock are difficult and expensive to prepare, and are therefore in short supply. The reader is referred to Section 3,B of this manual for suggested guidelines for the efficient preparation of reference standard solutions.

The repository stock is reviewed biennially and compounds for which there has been no demand or those which are no longer commercially produced are removed from the stock and replaced by pesticidal compounds more recently introduced to the marketplace. At the time of the stock overhaul a printed index entitled ANLYTICAL REFERENCE STANDARDS AND SUPPLEMENTAL DATA FOR PESTICIDES AND OTHER SELECTED ORGANIC COMPOUNDS is also updated, reflecting the stock overhaul. This booklet provides a complete list of all the compounds in stock along with some supplemental data such as chemical name structure, molecular weight, use, toxicity and an innovation introduced in the 1976 issue, literature references to residue analytical methodology for each compound if any could be located.

In preparing requests for standards, the requester is asked to list by code number and common name each compound needed. This assists repository personnel in processing requests, particularly those that are lengthy.

A final note is directed to all scientists associated with university laboratories. Requests for standards must be made on stationery bearing the letterhead of the institution and must be signed by a university official such as a department head. Pesticides will not be mailed to individuals submitting requests on personal stationery.

A special word of gratitude and apprecition is extended to pesticide manufacturers for their wholehearted cooperation in providing the repository program with analytical-grade standard materials at no cost to the program.

All requests for the current catalog and for standards should be directed to:

Quality Assurance Section, Analytical Chemistry Branch
EPA, Environmental Toxicology Divison (MD-69)
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

REVISIONS TO THE MANUAL

This manual is revised biennially and all persons on the mailing list will automatically receive copies of the revisions. The question then for each manual holder is whether his name is in fact on the list. Consider the following points:

1. If you received this Appendix section as part of a group of revisions, you are definitely on the list.
2. If you received this Appendix section as part of an entire manual you requested by mail or phone, you are definitely on the list.
3. If you received this Appendix section as a handout at some training course, and your name and affiliation were not recorded, you are probabaly not on the list and, therefore, will not automatically receive revisions.
4. If you obtained your copy of the Manual from some individual not associated with the Laboratory at Research Triangle Park, NC, you are probably not on the list and therefore will not automatically receive revisions.

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Date

Quality Assurance Section, Analytical Chemistry Branch
Environmental Toxicology Division
EPA, HERL (MD-69)
Research Triangle Park, NC 27711

This is to request that your record be reviewed to be certain the undersigned is on your mailing list to recieve copies of all future analytical manual revisions.

(Print or type name and full business address below)

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